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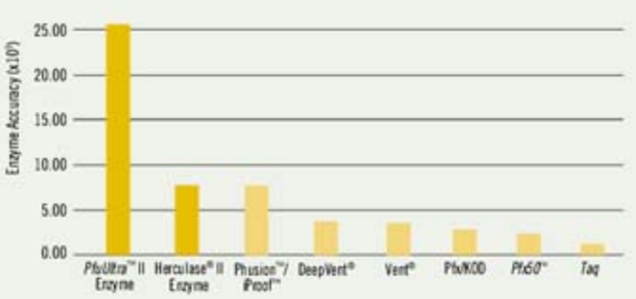

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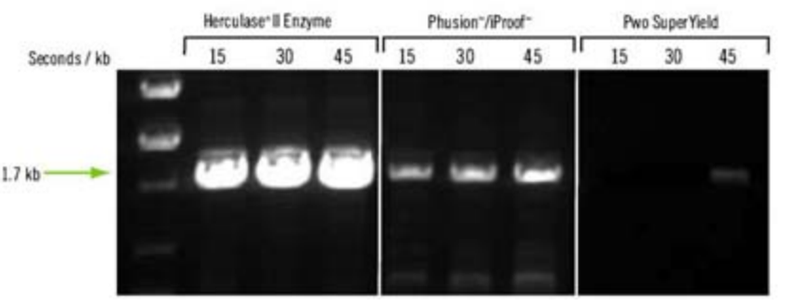

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GE05-07. First printed 05/2007.

Volume 316, Issue 5832

COVER

A female *Aedes aegypti* mosquito attempts to take flight after a blood meal. The complete sequencing of this disease vector is reported on page 1718, with an accompanying Perspective on page 1703.

Photo: James Gathany/CDC

DEPARTMENTS

EDITORIAL

NEWS OF THE WEEK

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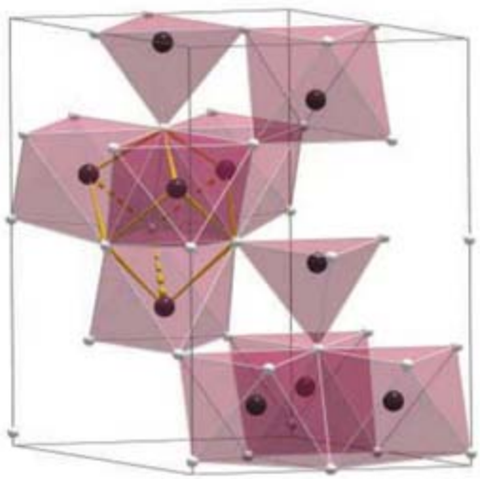

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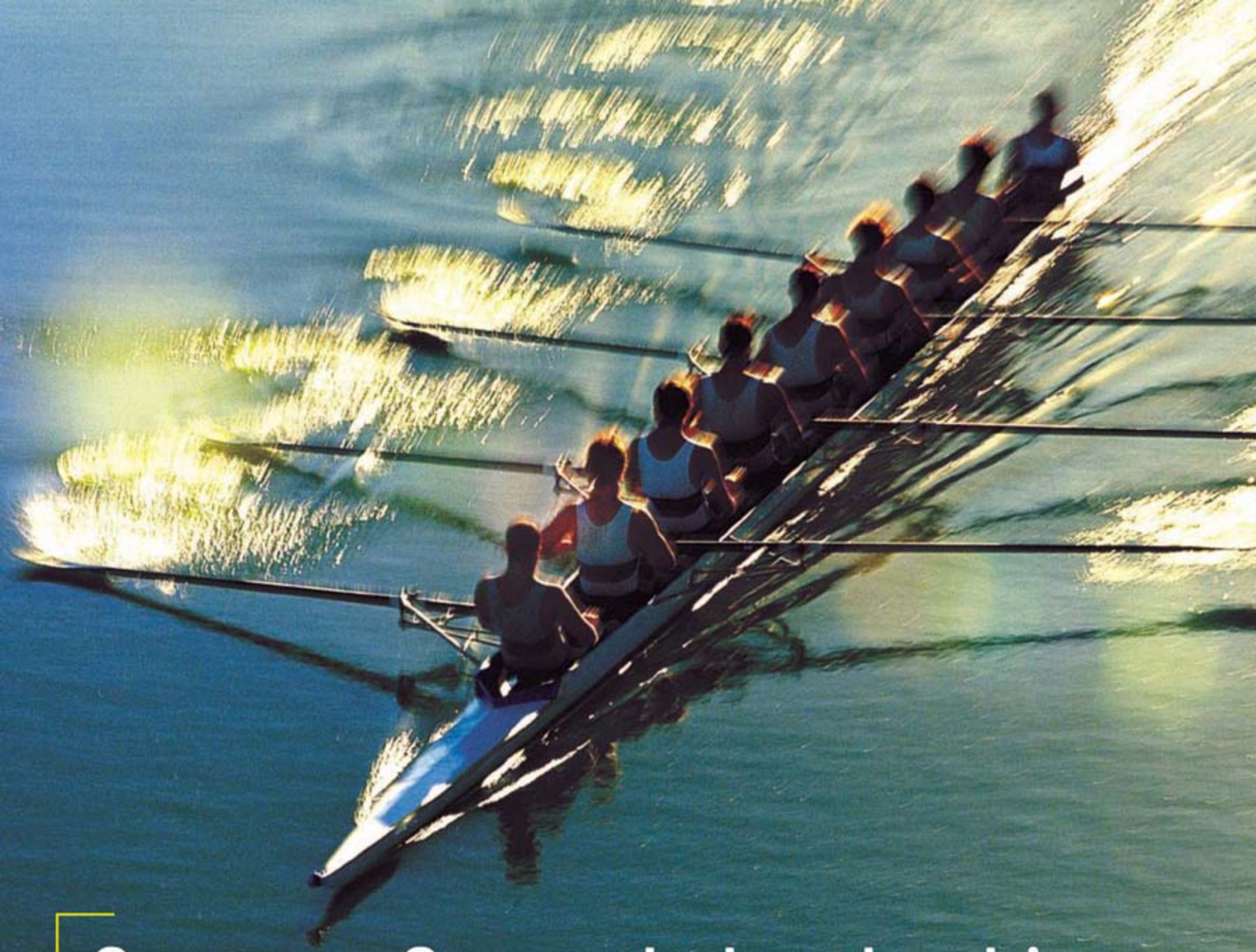
CONTENTS continued >>



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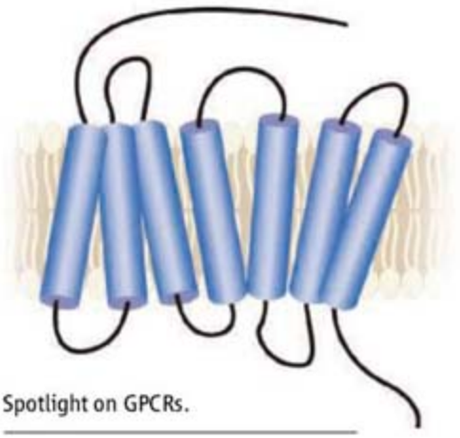

Spotlight on GPCRs.

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PERSPECTIVE: GPCRs Signaling Directly Through Src-Family Kinases
D. McGarrigle and X.-Y. Huang
G protein–dependent and –independent pathways couple seven-transmembrane receptors to nonreceptor tyrosine kinases.

PROTOCOL: A Cell-Free Scintillation Proximity Assay for Studies on Lysosome to Phagosome Targeting
V. Trivedi, S. C. Zhang, W. Stockinger, A. Nohturfft
This method allows the detection of the interaction between two different vesicular populations.

CREDITS: (SCIENCE NOW) RONGPING WEI, WOLONG GIANT PANDA RESERVE CENTRE; (SCIENCE CAREERS) COMSTOCK BUSINESS IMPACTS

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<< Toward Sustainable Fisheries

The currently observed crisis of overcapacity, overfishing, and accompanying problems of habitat destruction and bycatch, lies in competition among fishers. **Beddington *et al.*** (p. 1713) review how sustainable fisheries can be achieved if management strategies are adopted that remove subsidies and give individual fishers a right to a proportion of the total catch. By removing competition, fishers should then be prompted to act to sustain the entire fishery because, as the common fish stock improves, each individual's quota will increase. **Persson *et al.*** (p. 1743) studied fish stocks in Lake Takvatn in northern Norway, where a population of stunted Arctic charr of little worth to fisheries had replaced the mixed population of brown trout and charr. Fifteen years after the removal of 31 metric tons of fish, the size distribution of charr had shifted into a new steady state. More small fry were produced that made better prey for trout, whose population then recovered. Thus, culling of larger, slowly reproducing prey, rather than reintroductions of mature predators, might be effective in reversing an apparently collapsed fish stock.

Effective Translation

In large molecules, vibrational excitation of a particular bond often does not cause it to break because the spreading of vibrational energy across the entire framework is typically faster than bond-cleaving reactions. Smaller molecules, such as di- and triatomics, that have fewer vibrational modes are more likely to cleave particular bonds through vibrational excitation. **Yan *et al.*** (p. 1723; see the Perspective by **Crim**) have discovered that, contrary to this paradigm, when CHD_3 collides with Cl atoms to form CD_3 and HCl, translational energy promotes the reaction as effectively as selective excitation of the C–H stretch vibration. The study relied on precise control of the gas-phase collision energies, and suggests that even relatively small molecules have highly complex energy distribution dynamics.

Carbon Uptake Reconsidered

Approximately half of the CO_2 emitted by fossil fuel burning remains in the atmosphere; the rest is absorbed by the ocean or incorporated by the terrestrial biosphere in roughly equal measures. Two studies reassess the uptake of CO_2 by these sinks (see the Perspective by **Baker**). In order to understand the relative role of different parts of the terrestrial biosphere as carbon sinks, global measurements of atmospheric CO_2 concentration must be interpreted by "inversion" models to determine how uptake, emission, and transport contribute to the seasonal and regional differences. Previous studies have suggested that there must be a strong carbon sink in the Northern Hemisphere, and that the tropics are a net carbon source. **Stephens *et al.*** (p. 1732) report that global vertical distributions of CO_2 in the atmosphere are not consistent with that interpretation but are more consistent with models that show a smaller Northern Hemispheric carbon sink and possibly strong carbon uptake in the tropics. The rate of uptake of CO_2 depends on the difference between the partial pressure of CO_2 in the atmosphere and that which would exist if the ocean and the atmosphere were at equilibrium.

Le Quéré *et al.* (p. 1735, published online 17 May) report that the rate of uptake by Southern Ocean, one of the most important CO_2-absorbing regions, has slowed relative to what would be expected based solely on how fast the concentration of atmospheric CO_2 has risen since 1981. They attribute this shortfall to an increase in windiness over the Southern Ocean that increases the outgassing of natural CO_2. The increased windiness has also been ascribed to human activity, and the authors predict that this relative trend will continue.

Ferrihydrite Unfurled

The iron oxyhydroxide compound ferrihydrite, which is found in a wide range of natural sediments, is of interest for its effectiveness as a heavy metal scavenger in wastewater treatment, as well as its relation to biochemical iron sequestration motifs. However, its nanocrystalline morphology has impeded efforts to determine its precise structure. **Michel *et al.*** (p. 1726; see the Perspective by **Penn**; published online 24 May) have modeled pair distribution functions extracted from x-ray scattering data to obtain the lattice structure of the nanometer-scale particles. Their analysis supports a single hexagonal phase, with 80% of the iron ions occupying octahedral coordination sites in the ideal lattice.

Tropical Disease Vector Genome

The mosquito *Aedes aegypti* is responsible for the transmission of dengue and yellow fever, which together affect more than 50 million people each year. **Nene *et al.*** (p. 1718, see the cover and see the Perspective by **Chadee *et al.***) present the genome sequence for *Ae. aegypti*, which reveals extensive gene conservation with the malaria vector mosquito, *Anopheles gambiae*.

Continued on page 1667

CREDITS (TOP TO BOTTOM): PER-ARNE AMUNDSEN; BRITTON STEPHENS

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* For the purpose of this prize, molecular biology is defined as "that part of biology which attempts to interpret biological events in terms of the physico-chemical properties of molecules in a cell" (*McGraw-Hill Dictionary of Scientific and Technical Terms, 4th Edition*).

**Nobel Prize 2006 winners in Physiology or Medicine for their discovery of RNA interference – gene silencing by double-stranded RNA.

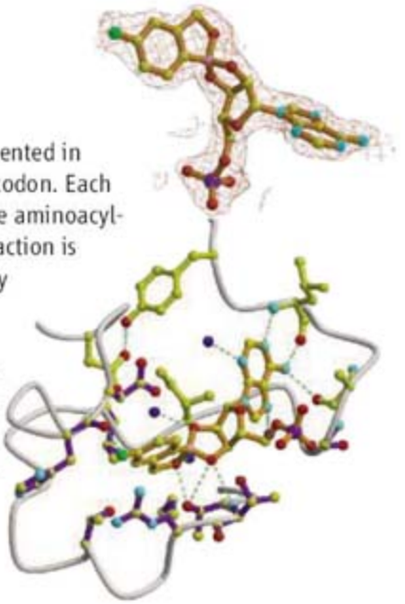
Continued from page 1665

As representatives of the two major mosquito subfamilies, the differences observed should underlie inherent biological traits including blood-feeding preferences, host-seeking behavior, and the ability to transmit specific pathogens. **Waterhouse *et al.*** (p. 1738) examined the evolution of innate immunity by comparing the genome of *Ae. aegypti* with the malaria mosquito, *An. gambiae*, and the fruit fly, *Drosophila melanogaster*, which represents a genetic outgroup. Different phases of immune signaling (recognition, modulation, signal transduction, transcriptional activation, and effector production) revealed different evolutionary dynamics.

In Support of Darwin

Darwin suggested that closely related species are less likely to coexist in communities because of shared resource requirements. Using a model plant-mycorrhizal system, **Maherali and Klironomos** (p. 1746) examined the effect of phylogenetic relatedness of mycorrhizae on roots of *Plantago* on the species richness that persisted 1 year after inoculation, on mycorrhizal production, and on productivity of the host plant. As predicted, community biodiversity was greatest when fungal species were more distantly related. Moreover, these species-rich communities had higher productivity than species-poor communities consisting of closely related taxa.

Boron Boost to Antifungal Agents



Transfer RNAs (tRNAs) recognize the genetic code represented in messenger RNAs, with tRNAs specific for each different codon. Each tRNA is charged with the correct amino acid by a cognate aminoacyl-tRNA synthetase (AARS). Because the accuracy of this reaction is vital in maintaining the fidelity of the genetic code many AARSs have evolved the ability to hydrolyze tRNAs aminoacylated with the incorrect amino acid, so-called "editing." **Rock *et al.*** (p. 1759) show that a benzoxaborole antifungal drug can inhibit yeast LeuRS by interfering specifically with the editing reaction. The boron atom in the oxaborole ring is critical for this effect, suggesting that incorporating boron into small molecule antifungals may lead to the production of additional classes of therapeutic agents.

Actin to Safeguard Transcription

Besides its well-characterized function as a cytoskeletal component, actin has emerged as a regulator of nuclear processes, including transcription. MAL, a coactivator of the transcription factor serum response factor (SRF), directly binds to and senses cellular levels of monomeric G-actin. MAL responds to serum-induced depletion of cellular G-actin with nuclear accumulation and SRF activation. **Vartiainen *et al.*** (p. 1749; see the Perspective by **Wu and Crabtree**) report that MAL rapidly shuttles between the nucleus and cytoplasm in resting cells. Actin binding in the nucleus targets MAL for efficient nuclear export and, furthermore, prevents activation of SRF during the short time that MAL spends in the nucleus. When growth factor stimulation interferes with actin binding, this lock on MAL activity is released.

Resurrecting Infections Past

Our genomes contain endogenous retroviruses that can be regarded as an archaeological record of past infections. **Kaiser *et al.*** (p. 1756) undertook a genetic excavation to explore why chimpanzee and gorilla genomes, but not those of humans, contain hundreds of copies of a particular endogenous retrovirus. They revived the virus's core protein from the chimp genome and found that infection of cells with chimeric viruses containing this protein could be inhibited by the human antiviral factor, TRIM5α. However, the same protein had relatively poor activity against human immunodeficiency virus type 1 (HIV-1), contrasting with the efficiency primate TRIM5α against both viruses. It seems that by acquiring resistance to one ancient virus, humans became more susceptible to HIV-1.

CREDITS: ROCK *ET AL.*

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Donald Kennedy is Editor-in-Chief of *Science*.

Fixing the Drug Laws

THE PHARMACEUTICAL BUSINESS IS A MULTINATIONAL ENTERPRISE OF GREAT SIGNIFICANCE to human health. The U.S. Food and Drug Administration (FDA), once called the world standard in regulating new drug approvals, may still deserve that status. But, as was described earlier in this space, public confidence in the agency has been shaken. That's why it's important that we restore the FDA's capacity to do the quality of work that all health care systems require.

The immediate opportunity to fix things up lies with Congress. A bill authored by Senator Edward Kennedy has passed the Senate. Last month, three former FDA commissioners (myself included) testified before Rep. Henry Waxman's House Oversight Committee and called for stronger budget support, noted problems with the "user fees" mandated by earlier legislation, and cited the growing rate of antibiotic resistance. We also pleaded for improved monitoring of the safety of marketed drugs, already a troubling source of public concern.

With respect to the last of these, the problem is that the United States lacks a system that is adequately tuned to detect adverse reactions. That measure requires a numerator and a denominator: the number of reported adverse events divided by the number of prescriptions issued. The FDA knows neither. Event reporting is voluntary, yielding a record of dubious reliability, and there's no national prescription record. That's why the FDA had to use Kaiser, a large health maintenance organization, to find an adequate database for evaluating the safety of Vioxx.

Information about all clinical trials reviewed by the FDA should be made publicly available at the FDA's Web site and also be linked to the National Institutes of Health's Web site, ClinicalTrials.gov. Why? Recently, a Cleveland Clinic cardiologist analyzed trials conducted on GlaxoSmithKline's diabetic drug Avandia. His meta-analysis revealed cardiovascular risks that had been hinted at in earlier published trials but were not statistically significant in any one trial. The study, published in the *New England Journal of Medicine*, illustrates the difficulty of finding important trial data. The journal has since been attacked by the company, some physicians, and an unfortunate editorial in the *Lancet*, but it deserves praise instead.

On antibiotic resistance, a new report from Resources for the Future (RFF) entitled "Extending the Cure" lays out the contemporary hazards. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci are widespread in hospitals. The RFF authors note that deaths from the 1918 influenza epidemic were largely from untreated infections—a chilling prospect as we await possible repetition of such an epidemic. The new legislation should provide positive incentives for drug companies to develop new antibiotics, but physicians also need to recognize that their own prescribing behavior exacts external costs on the health care system.

Merely applying policy fixes for these deficiencies can't solve the biggest FDA problem. It's about resources. Consider: In 2003, the first fiscal year after 9/11, the FDA's budget benefited from the Department of Homeland Security. That year's budget would have grown to $1.924 billion in 2007, had it received annual increases of 5.8% to meet the real costs of inflation. Instead, what the FDA actually got was $1.558 billion, 20% below the agency's needs. In its next budget, the FDA needs $2 billion in appropriated funds even to stay level with 2003.

The 1997 legislation mandating user fees got the agency more money, but generated public doubt about the FDA's relationship with industry. The House could pay more attention than the Senate to another problem: User fees can be applied only to the drug approval process, where the new appointments exact costs from the rest of the FDA. Not only are those fees unavailable for drug safety monitoring, they can't be applied to food safety either (think of *Escherichia coli* in spinach and tainted meat).

Beyond addressing the FDA's funding deficit through appropriations rather than user fees, the House should make "orphan drug" provisions clearly available for developers of new drugs that confront antibiotic resistance. Its bill should be firm about requiring public availability of all clinical trial results and include muscular provisions for monitoring drug safety, such as a national database that contains required reports of adverse drug reactions and provisions for a national prescription audit. The latter may be politically naïve, but why take essential needs off the table because they may not be politically popular?

– Donald Kennedy

10.1126/science.1145966

EDITED BY GILBERT CHIN AND JAKE YESTON

ENVIRONMENTAL SCIENCE

Fish Fatalities While Feeding

Despite the accumulated evidence that rapid climate change has deleterious effects on a broad range of animal populations, there are few data indicating how these effects are mediated. Biro *et al.* conducted a field experiment by stocking nine small lakes in British Columbia with trout in the warm summer of 1998 and in the cool summer of 1999. They found that water temperatures above 17.5°C increase the metabolic rate of young rainbow trout, so in order to maintain their rate of growth, the young fish compensate by feeding more actively. It became apparent that the increased feeding activity increased the young trout's visibility to predators (usually adult trout), such that the survival of the young in a warm year was only half that in a cooler year. Hence, the small increases in water temperature (of just a few degrees) caused by climate change will substantially reduce the survival of lake trout populations for which escape by migration is not possible. — CA

Proc. Natl. Acad. Sci. U.S.A. **104**, 9715 (2007).

CHEMISTRY

Droplets Hanging Around

Microdroplets of common liquids deposited on surfaces typically evaporate in minutes if the surrounding ambient is not saturated in the corresponding vapor phase. Cheng *et al.* observed that microdroplets of 1-propanol and water in a 3-to-2 volume ratio showed remarkable stability when deposited on 1-decanethiolate monolayers self-assembled on gold surfaces. After initially undergoing evaporation to a volume of 0.2 μl, these microdroplets could persist for up to 5 hours. Variation of the volume ratio or deposition on substrates such as glass or polycarbonate led to rapid evaporation. The authors propose that the unusual stability results from the more volatile alcohol segregating to the bottom of the microdroplet near the hydrophobic alkyl chains, and the outer water-rich layer deriving stability from formation of a maximally hydrogen-bonded network. — PDS

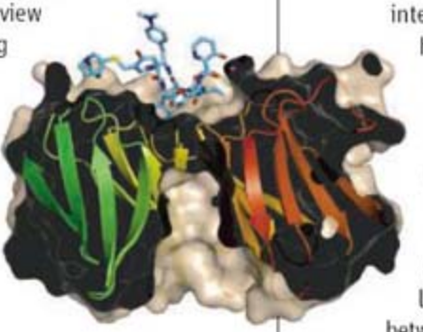
J. Phys. Chem. B **111**, 10.1021/jp069063f (2007).

BIOCHEMISTRY

Bacterial Drug Design

The most effective weapons for fighting bacterial infections are those that bacteria use against each other. One tactic for combating the spread of drug-resistant strains is to use multiple drugs, such as the combination of dalfopristin (a type A streptogramin) and quinupristin (a type B streptogramin). The former blocks an early step in ribosomal protein synthesis, whereas the latter blocks a late step.

Korczynska *et al.* describe the crystal structure of virginiamycin B lyase (Vgb) in complex with quinupristin (fortuitously, a chock of dalfopristin immobilizes two Vgb molecules in the crystal, but this interaction is without an in vivo correlate). Vgb inactivates the cyclic peptide antibiotic by catalyzing a linearization, and structure-based mutagenesis supports the mechanistic proposal that ring opening occurs not via hydrolysis of an ester but by means of a C-O lyase reaction. The detailed view of quinupristin binding to Vgb is consistent with its versatility in detoxifying natural and semisynthetic type B streptogramins—the known modifications all point into the solvent and away from the active site. A comparison of this complex with that of quinupristin bound to the 50S ribosomal subunit may guide design efforts aimed at reducing its affinity for Vgb without lessening its ardor for the ribosome. — GJC



The structure of Vgb.

Proc. Natl. Acad. Sci. U.S.A. **104**, 10388 (2007).

CLIMATE SCIENCE

Early Reversals

Over the Pleistocene epoch, sea level was more than 100 m lower during some glacial periods than it is now; even within cold intervals, it may have varied by tens of meters. During the last interglacial, global average temperatures were near where they are expected to be in the coming century, and sea level was 4 to 6 m higher. Thus, conditions in that period seem relevant to our near future. Recently compiled evidence suggests that sea levels fluctuated by as much as 30 to 40 m during the beginning of that warm interval, but the large changes inferred have been controversial due to a lack of corroborating records. Andrews *et al.* have confirmed the variability using deposits that record the relative elevations of the Greek shoreline. By precisely determining the sample ages via U/Th dating, they found that sea level twice dropped precipitously between 136,000 and 135,000 years ago, near the end of deglaciation, an observation that supports earlier findings from the Red Sea and from Papua, New Guinea. Their results also help to constrain the timing of sea-level rise during the penultimate deglaciation. — HJS

Earth Planet. Sci. Lett. 10.1016/j.epsl.2007.05.005 (2007).

CREDITS (TOP TO BOTTOM): P. BIRO; KORCZYNSKA *ET AL.*, *PROC. NATL. ACAD. SCI. U.S.A.* **104**, 10388 (2007)

CHEMISTRY

A Chain to Break Nitrogen

Synthetic chemists continue to puzzle over how bacteria manage the feat of reducing triply bonded nitrogen to ammonia without the help of extreme temperatures or pressures. Among the clues teased out of nitrogenase enzyme studies is the possible involvement of paramagnetic iron hydride centers. However, low-valent iron model compounds tend to be diamagnetic. Sadique *et al.* have applied a sterically bulky β-diketiminate ligand (L) that, despite differing structurally from the sulfur ligands in the enzyme, does coordinatively stabilize high-spin Fe(II) hydrides. Moreover, like nitrogenase, the resulting model complexes can fully cleave an N=N double bond. Through a series of careful experiments, the authors explored the mechanism whereby two of these paramagnetic LFe-H centers split azobenzene (PhNNPh) to yield LFeNHPh complexes. They showed first that reaction with excess azobenzene leads to an isolable intermediate, LFeN(Ph)NHPh (shown above), which on heating is transformed into LFeNHPh and half an equivalent of azobenzene. Kinetic studies on this latter step revealed it to be first order in iron, after an induction period, which ruled out a bimolecular pericyclic rearrangement to the products. Moreover, the intermediacy of LFe-H (stemming from β-hydride elimination) was inconsistent with trapping studies. The data were most consistent with a radical chain mechanism initiated by homolytic dissociation to LFe and PhNNHPh, a hypothesis supported by disappearance of the induction period on addition of the Fe(I) complex K[LFeCl] to the reaction mixture. The results argue for deeper consideration of single-electron chemistry in probing the enzyme mechanism. — JSY

J. Am. Chem. Soc. **129**, 10.1021/ja069199r (2007).

GENETICS

Finding Bigfoot

The presence of conserved noncoding regions in genomes is a footprint that points to the likely existence of conserved modes of gene regulation. In plants, there appear to be fewer conserved noncoding sequences than in animals; because they are shorter, they are harder to find. Freeling *et al.* examined the genome of *Arabidopsis*, which underwent a duplication of its genome (the α event) in its recent history, in order to identify noncoding sequences that border genes and that have been retained. Large regions, relatively rich in conserved noncoding sequences (such as the G-box CACGTG), were designated Bigfoot and were often associated with transcription factor binding motifs. Smaller regions of noncoding sequences were also identified (and dubbed Smallfoot) and were often linked to components of signal transduction pathways. Few of these noncoding sequences were identified outside of paralogous genes, suggesting that the regulatory regions of other genes are less conserved or may evolve at a rapid rate. — LMZ

Plant Cell **19**, 10.1105/tpc.107.050419 (2007).

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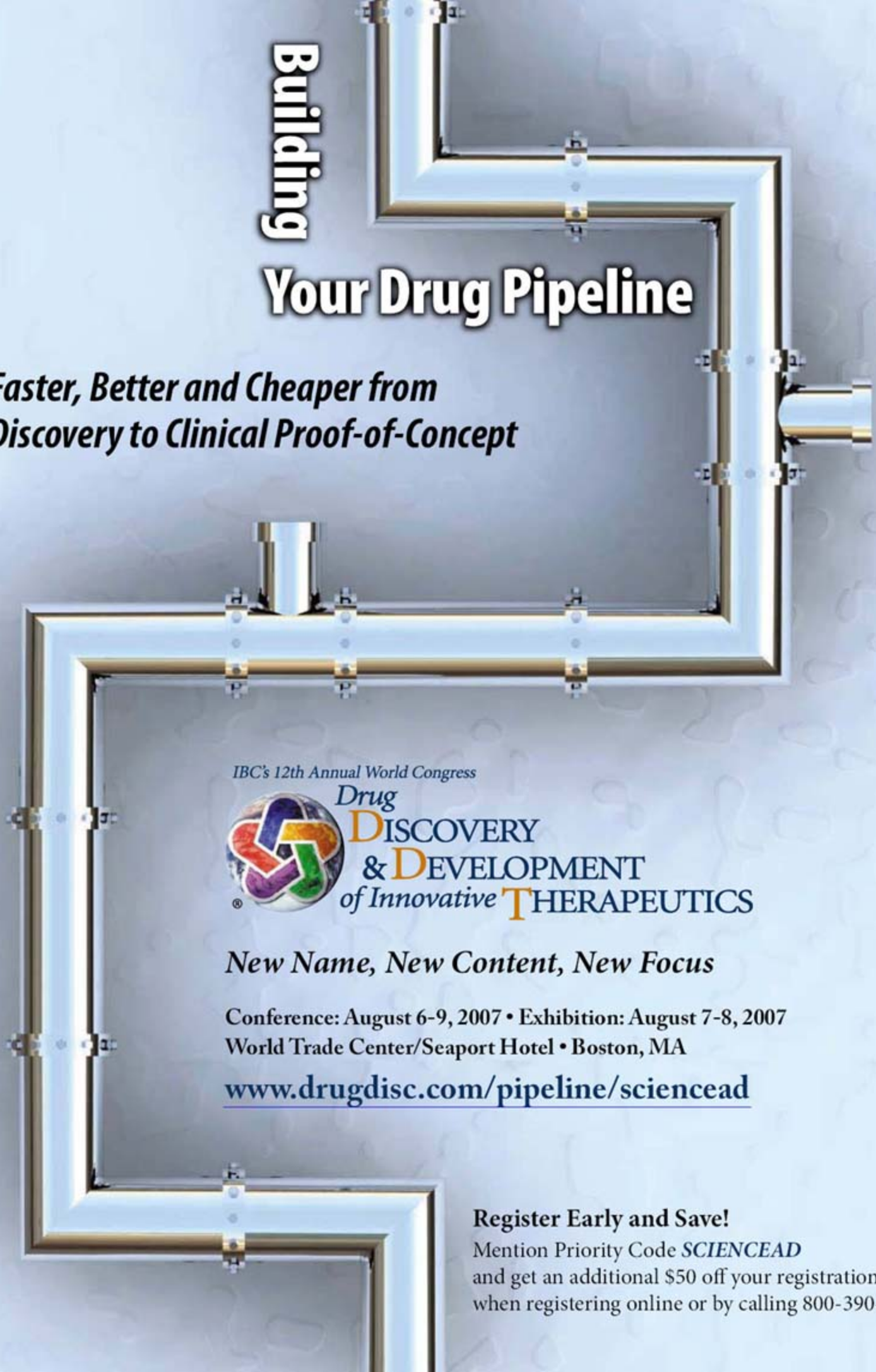

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Crumpling: A New Wrinkle

Colleagues of Marcelo A. F. Gomes, a physicist at the Federal University of Pernambuco in Recife, Brazil, can fairly accuse him of skimming the cream off the top in his research. In an unusual experiment, Gomes and his team have studied

Crumpled cream.

how films of cream that form on heated milk crumple when they are hoisted from the liquid and set down on a glass plate.

Even as a child, Gomes says, he was fascinated by the films on his café au lait, which would crumple whenever he tried to pluck them off. Others have studied how paper and polymer sheets crumple, Gomes says, but cream is different: The film is so flimsy it will wad up under its own weight.

The wads are neither two-dimensional sheets nor three-dimensional solids. By measuring them and peering inside with nuclear magnetic resonance, the researchers found that the wads have a "fractal" dimension of about 2.5, as they report in the 21 June issue of the *Journal of Physics D*. Because that dimension is the same as that for crumpled paper, the experiment shows that what matters is not how you squash the wads but how their creases and crinkles fit together, says Sahraoui Chaieb, a physicist at the University of Illinois, Urbana-Champaign: "It's all geometry."

Standardizing Stem Cells

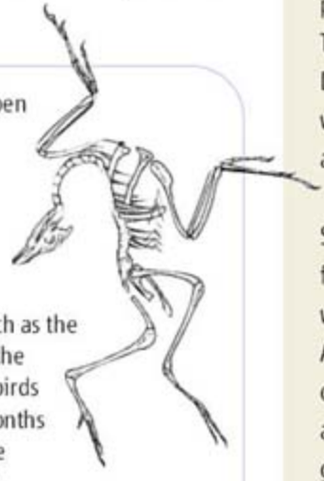
Stem cell researchers from around the world have taken a big step toward imposing order on the rapidly spreading landscape of human embryonic stem cell research. In the current issue of *Nature Biotechnology*, researchers from 17 labs in 11 countries report that they now have comparable data on 59 cell lines.

The study is part of the International Stem Cell Initiative, headed by Peter Andrews of the University of Sheffield, U.K. "The object was to get everyone to grow cells in as standard conditions as we could," says Andrews.

In phase 2 of the initiative, scientists will compare various media for growing cell lines and will analyze gene changes over time—particularly relevant for the lines approved for U.S.–funded researchers, which are the oldest of the lot. A registry, with recipes to enable researchers to obtain comparable data from new cell lines, will be maintained at the Web site of the International Stem Cell Forum, the U.K.–based group that is funding the studies.

Dino Death Throes >>

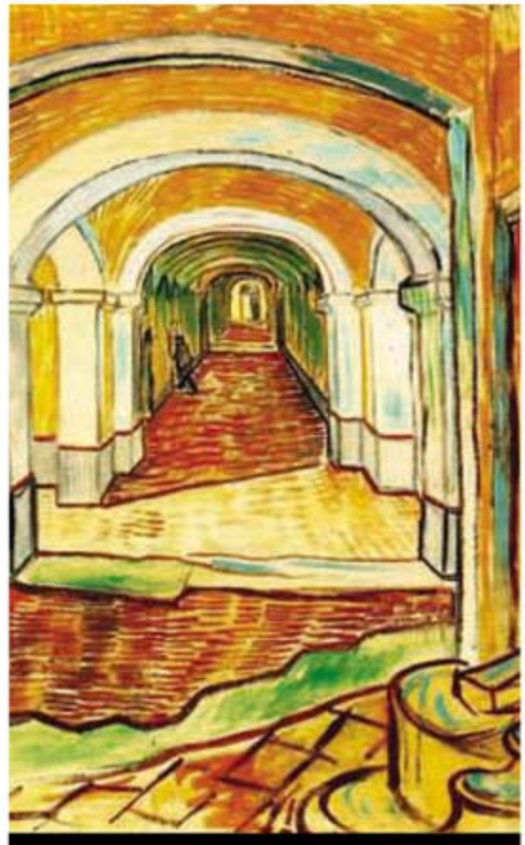
Many fossilized dinosaurs are found in a dramatic death pose: wide-open mouth, thrown-back head, tail twisted over the body. Most scientists have assumed that the posture is caused by events after death. But paleontologists Kevin Padian of the University of California, Berkeley, and Cynthia Marshall Faux of the Museum of the Rockies in Bozeman, Montana, believe it reflects death throes from central-nervous-system trauma.



Archaeopteryx.

"Most traditional interpretations … of the 'dead bird' posture"—such as the effects of drying muscles or water currents—"explain few or no cases," the authors say. They reached that conclusion after monitoring newly dead birds to see the effects of rigor mortis. They also dried red-tailed hawks for months as their muscles and ligaments shriveled up. In neither case did the pose develop, the authors report in the current (March) issue of *Paleobiology*. Rather, they suggest other causes such as poisoning or suffocation. They point out that the death pose has been found in dinosaur and bird fossils in northeastern China's Jehol biota, where animals might have been asphyxiated by volcanic gases.

"I am pleased to see experimental support brought to bear on this question," says paleobiologist Matthew Carrano of the Smithsonian Institution in Washington, D.C. "I don't think every dinosaur in this pose will necessarily have died in this manner, but [now] we have more possible explanations to choose from."



ILL LITERACY

NET WATCH

From sickly Tiny Tim in Charles Dickens's *A Christmas Carol* to the disfigured character in the movie *The Elephant Man*, illness and its consequences have preoccupied writers, painters, and filmmakers. The Literature, Arts, and Medicine Database aims to help students use these works to understand disease, health care, and the social issues they raise.

The site from the New York University School of Medicine catalogs hundreds of films, paintings, novels, and other titles with medical connections. Tuberculosis, AIDS, and mental illness have drawn plenty of interest over the years; diabetes and arthritis, much less. Commentaries by guest scholars elucidate works such as Vincent van Gogh's painting of the mental asylum where he spent much of his final year of life (above). The barren hallway—the only figure is fleeing—reflects his isolation during his illness. >>
litmed.med.nyu.edu

CREDITS (TOP TO BOTTOM): VINCENT VAN GOGH; R. EMMANUEL DE SOUZA/FEDERAL UNIVERSITY OF PERNAMBUCO; C. M. FAUX AND K. PADIAN (2007)/*PALEOBIOLOGY* **33**: 201–226, FIGURE 1A

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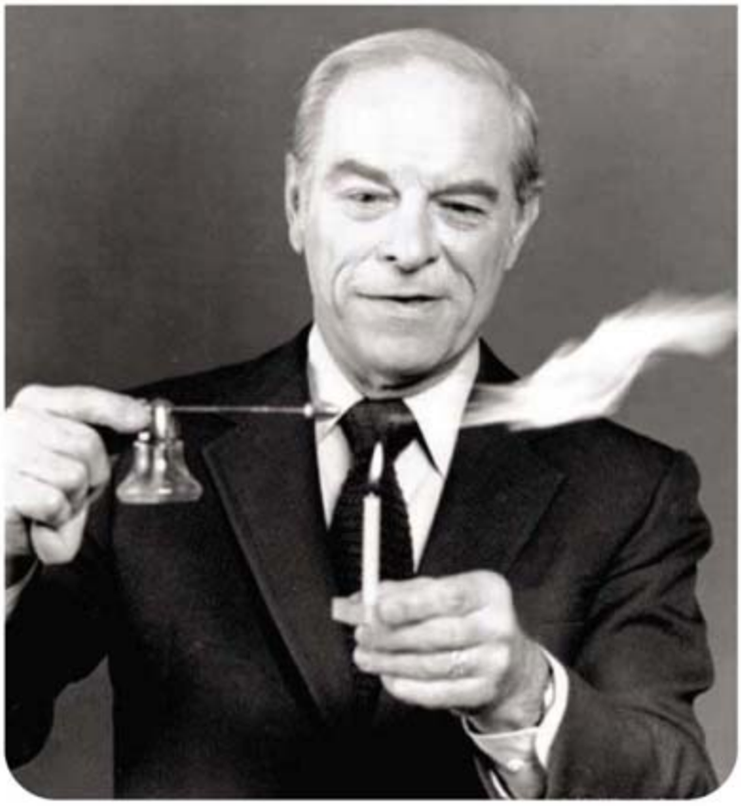

DEATHS

THE WIZARD. Don Herbert, who died 12 June at age 89, never got to put "Dr." in front of his name. Nonetheless, he helped jump-start thousands of careers in science as television's Mr. Wizard, reaching a national audience starting in the 1950s with his own show and appearances on other programs as well as through radio, books, and magazines.

Herbert prepared, in a way, for doing science on live TV by majoring in English and general science in college and performing in school theatre. His mastery of the medium was evident whether he and his child guest were using atmospheric pressure to crush a can, timing the speed of a cockroach, or finding all the types of energy in a Rube Goldberg contraption.

"I was awed by him," says chemistry educator Bassam Shakhashiri of the University of Wisconsin, Madison, who first saw *Watch Mr. Wizard* when he arrived in the United States from Lebanon as a college student. "It came across that he was himself learning and enjoying it. He's had a lasting effect on kids of all ages."

CAMPAIGNS

DONE WITH THIS. The head of the soon-to-close Savannah River Ecology Laboratory (SREL) is making a last-ditch attempt to save the lab by resigning. Paul Bertsch hopes that his departure will help persuade the lab's sponsor, the Department of Energy (DOE), to reconsider its decision to turn off funding for the University of Georgia (UGA)–run lab at the end of the month (*Science*, 18 May, p. 969).

"The quest for truth and justice often comes at a price," Bertsch wrote staffers last week, after a month of phone calls and public bashing of both the federal agency and the university, which could also bail the lab out. He says he wasn't asked to leave. SREL population ecologist David Scott says he and his colleagues think Bertsch did the best he could. "He's been kept out of the loop," Scott says.

Bertsch, a tenured UGA biogeochemist, would like to return to his onsite work in radionuclide transport and nanoparticles, "providing SREL is here." He says he's heartened by a congressional inquiry into the matter and positive responses by agencies including the National Nuclear Security Administration to funding requests by SREL researchers.

Got a tip for this page? E-mail people@aaas.org

IN THE NEWS

NO FOUL PLAY. When Pakistan's cricket coach, Bob Woolmer, was found dead in his hotel room in Jamaica during the World Cup in March, a government pathologist concluded that he had been strangled. The subsequent investigation put several Pakistani cricket stars under the scanner. Now Jamaican authorities say that the pathologist who conducted the autopsy, Ere Sheshiah, was mistaken and that Woolmer died of natural causes, possibly heart failure. The new ruling is based on work by three pathologists in the United Kingdom, South Africa, and Canada.

"There was no evidence to support manual strangulation," says Michael Pollanen, chief forensic pathologist for Ontario, who reviewed the autopsy report, digital images of the body, and other evidence. The bruising on Woolmer's neck, initially seen as evidence of foul play, is a "mimic of strangulation" and most likely occurred during the autopsy, Pollanen says.

Sheshiah, however, is standing by his report that the coach was murdered, according to his comments in the *Jamaica Observer*.

Awards >>

FAULTFINDING. Over several decades, geophysicist Hiroo Kanamori has plowed through reams of analog earthquake data to clarify the basic fault-rupture processes of big earthquakes. What he found led to the development of countermeasures that mitigate earthquake damage. Last week, his efforts were rewarded with one of three Kyoto Prizes awarded by Japan's Inamori Foundation.

"I admire his courage in working on complicated problems," says Robert Geller, a geophysicist at the University of Tokyo who first met Kanamori as an undergraduate in the early 1970s at the California Institute of Technology in Pasadena, where Kanamori is now a professor emeritus. Although theories and methods existed to analyze the analog records of big earthquakes, Geller says, researchers shied away from the nitty-gritty work. "Hiroo rolled up his sleeves and got to work."

Other Kyoto Prizes this year went to Hiroo Inokuchi, a professor emeritus at the University of Tokyo, for his pioneering research on organic materials that paved the way for organic molecular electronics, and to German choreographer Pina Bausch, for exploring "the fundamental motives of human action." Each laureate receives a gold medal and $410,000.

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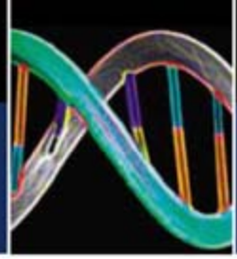
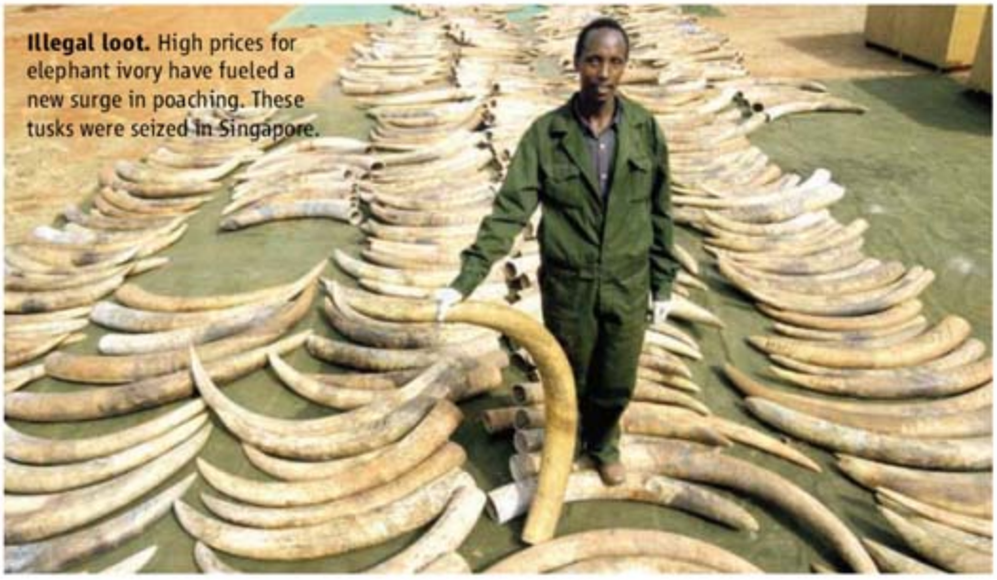


Regulating synthetic biology 1682

National medals' gender imbalance 1683



Illegal loot. High prices for elephant ivory have fueled a new surge in poaching. These tusks were seized in Singapore.

ENDANGERED SPECIES

Elephants Take Center Ring at CITES

Africa's elephants won a 9-year reprieve at the recent meeting of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Delegates from 171 nations at the 2-week conference in The Hague, the Netherlands, dealt with trade issues affecting a host of species, including corals (see sidebar, below), rhinoceroses, tigers, and leopards. But nothing was as contentious as the debate over elephants and their ivory, which began the first day and was settled the day before the meeting's close—and then only after several cabinet-level ministers from the key African countries took over the reins from their delegates. It's the first time at a CITES meeting that such political muscle has been used to hammer out an agreement.

Although CITES banned the ivory trade in 1989, it has since agreed to list elephant populations in South Africa, Botswana, Namibia, and Zimbabwe on Appendix II, permitting controlled sales of tusks collected from elephants that died of natural causes or in culling operations and from poaching seizures. In exchange for the pause in this trade, which Kenya and Mali insisted on, the deal permits the four southern African states to sell raw ivory from stockpiles registered with their respective governments as of 31 January 2007. The exact tonnage has not yet been determined, although estimates are between 100 and 200 metric tons. This ivory will be added to an additional 60 metric tons from South Africa, Botswana, and Namibia that CITES previously approved for a one-off sale. Japan is the sole CITES-approved country for the ivory trade, based on its import controls, although China is lobbying hard to be similarly recognized. All proceeds from the sales must be used for elephant and community-based conservation.

"Some call this a win-win," says Will Travers, president of the Species Survival Network in Washington, D.C. "But the true test will come with what happens to elephants on the ground." Adds Michael Wamithi, a wildlife biologist from the International Fund for Animal Welfare in Nairobi, Kenya, and former director of the Kenya Wildlife Service: "These two sales will put a huge amount of ivory into the Japanese market, igniting a high demand for ivory, which the legal market will be unable to sustain. That means more poaching."

Indeed, poaching and illegal ivory trade are already on the rise, say several researchers, basing their claim on what they see on the ground and the increasing tonnage of confiscated illegal ivory. "Any legal trade in ivory stimulates a parallel illegal trade," asserts Iain Douglas-Hamilton, an elephant researcher in Kenya with Save the Elephants. Following the 1989 ban on trading ivory, poaching "stopped overnight." It quickly resumed, he and others say, when CITES agreed in 1997 to permit Botswana, Namibia, and Zimbabwe to sell 50 metric tons.

But Tom Milliken, director of Traffic, the World Conservation Union's (IUCN's) wildlife monitoring network, disputes the idea that the legal trade leads to poaching. "From 1999 to 2004, there was a downward trend in illegal ivory seizures," he says, drawing on the data from IUCN's Elephant Trade Information System. But the trend shot upward. Twenty-five thousand kilograms of ivory were seized beginning in August 2005. That's more ivory than was seized in the previous 3 years combined, triggered, researchers say, by a surge ▶

CORALS: SUFFERING FROM WHIPLASH

What a difference 48 hours makes: On 13 June, delegates to the Convention on International Trade in Endangered Species voted to list all species in the genus *Corallium* (pink and red corals) in Appendix II, which limits trade. But on 15 June, after the conference was scheduled to end, they voted by secret ballot to reverse that decision, leaving the jewel-like colonies to the mercy of the coral hunters who scrape the sea floor with heavy trawlers for their prey.

Red corals are one of the most valuable wildlife commodities, with a finished necklace costing $20,000 or more. Over the past 2 decades, red coral harvests have dropped by 90% because of overcollecting, a problem the Appendix II listing was intended to correct. "These animals are sitting ducks on the sea floor," fumes Elliott Norse, president of the Marine Conservation Biology Institute in Bellevue, Washington, one of many outraged scientists. Norse compares the trawling method of harvesting corals to "clear-cutting a forest as a way to get a couple of ginseng plants." Studies indicate that coral populations never fully recover from the trawling.

After several delegates had left for home, Tunisia, Algeria, and Morocco—all coral-exporting countries—moved to reopen the debate and called for the secret ballot. This time, the resolution to protect the *Corallium* failed to gain the necessary two-thirds majority. "Obviously, there's something wrong with an organization that makes a decision and then unmakes it—after the meeting is over," says Norse. **–V.M.**

CREDIT: IFAW/D. WILLETTS

Megafish in trouble
1684

New era for bald eagles
1689

in the price, which is now roughly $850 per kilo; soaring demand for ivory in China and Japan; lack of law enforcement; and the involvement of organized crime.

Legal sales provide cover for the illicit trade, argue Douglas-Hamilton and Samuel Wasser, a conservation geneticist at the University of Washington, Seattle, because after the ivory leaves Africa there are no controls to prevent it from being sold as "legal" ivory. It was also almost impossible to pinpoint where it came from. "It was like a black box, but we've finally pried it open," says Wasser, referring to the DNA fingerprinting technique he's developed to trace illegal ivory back to its country of origin.

Last year, Wasser used this tool to track 531 tusks seized in Singapore, and representing about 1000 elephants, to Zambia. Zambia has not been authorized by CITES to trade ivory. "This is just the tip of the iceberg," says Wasser, noting that law enforcement officials estimate that only 10% of illegal ivory shipments are intercepted. Based on his calculations, ivory from 37,700 elephants is now entering the market illegally each year. "The poaching is worse than in the late 1970s," he says, when there were roughly 1.3 million elephants in Africa. Poaching reduced that number to 600,000 by 1989 when the full ivory ban was enacted. "Today, there are 470,000—and we're losing 8% a year. That's not sustainable."

Wasser's technique, presented in a paper at CITES, may help reduce poaching, says Douglas-Hamilton, "since it eliminates any speculation about where the ivory came from" and can be used to help track the criminals involved. He and other scientists agree that the main source of today's illegal ivory is the Congo Basin, where the forest elephants (*Loxodonta africana cyclotis*) are in sharp decline.

Whether the 9-year "resting period," as CITES has labeled the ivory-trade pause, will help African elephants overall is unclear. Because elephants don't reach sexual maturity until they're 12 years old, "it would have made sense scientifically if it had been a 24- or 36-year ban," says Rudi van Aarde, a conservation ecologist at the University of Pretoria in South Africa. "So this was a political decision."

Conservation scientists did celebrate when China voted with the rest of the 170 nations to stop raising captive tigers except for conservation purposes and to phase out its commercial farms, which raised the cats in hope of a domestic trade in tiger parts. For the tiger, which experts say is on a "catastrophic" path to extinction, that was an undisputed win-win.

–VIRGINIA MORELL

Virginia Morell is a writer in Ashland, Oregon.

CLINICAL RESEARCH

No Lifeline for Proposed Breast Cancer Prevention Trial

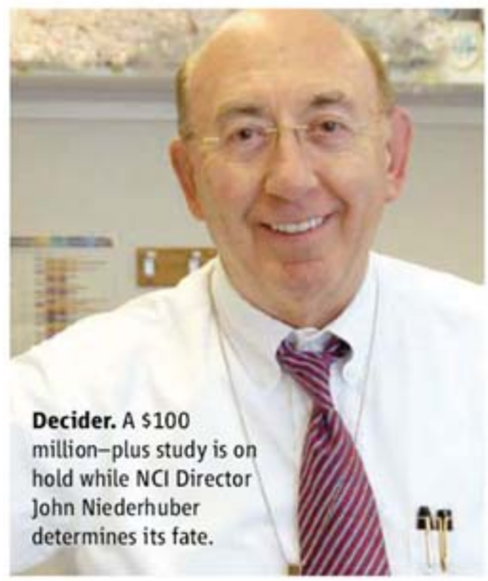
What was planned as one of the largest-ever U.S. breast cancer prevention trials may be scrapped after getting a lackluster review. Although the panel used mild words—it declined to "offer strong endorsement" for funding—the judgment last week by a subcommittee of the National Cancer Advisory Board (NCAB) seems likely to kill the $100 million study, which would test a new drug against an older one for high-risk women.

National Cancer Institute (NCI) Director John Niederhuber had not issued a decision earlier this week. But his opposition in the past suggested he was ready to pull the plug. Meanwhile, Niederhuber must weigh 2000 letters to Congress, as well as one to himself from Senator Arlen Specter of Pennsylvania, the ranking Republican on the subcommittee that draws up NCI's budget, supporting the trial. The trial's leaders at the National Surgical Adjuvant Breast and Bowel Project (NSABP) in Pittsburgh, Pennsylvania, declined to comment.

Known as STELLAR, the trial would compare a new drug called letrozole with an older drug to prevent breast cancer in 12,800 high-risk but healthy postmenopausal women. STELLAR had been reviewed seven times within NCI and by outside peer reviewers before Niederhuber put it on hold, questioning its scientific value and cost (*Science*, 16 March, p. 1477). At his request, an NCAB ad hoc panel met with other experts and patient groups to review the trial behind closed doors on 23 March.

Although panel members had "often divergent views," their "dominant opinion [was] that, because of concerns about toxicity, [the trial's] effect on the practice of preventive medicine might be modest," according to a report released last week at an NCAB meeting. Side effects have likely already deterred women from using other breast cancer preventive drugs such as tamoxifen, the report says. The three-member NCAB subcommittee noted that after NSABP's initial 5-year cost of $55 million, follow-up could require $80 million.

Peter Greenwald, director of NCI's Division of Cancer Prevention, made an impassioned plea for the trial, arguing that it "should be a top priority of NCI" because it could prevent 70% of breast cancer incidence in women at high risk. Some NCAB members were sympathetic, noting that NSABP leads the field, although they also recognized that NCI has a budget problem. But cancellation could cause "a collapse of the network" that has run breast cancer prevention trials for 15 years, warns participating investigator Patricia Ganz of the University of California, Los Angeles.



Decider. A $100 million–plus study is on hold while NCI Director John Niederhuber determines its fate.

CREDIT: MARTY KATZ/SCIENCE

At last week's NCAB meeting, Niederhuber also confirmed that a predicted 10% cut in the 2007 budget for cancer clinical trials would not happen after all. Warned to prepare for such a cut last year, the U.S. cooperative groups reduced enrollment by 3000 patients and delayed or canceled many trials (*Science*, 2 March, p. 1202). Group leaders say most of the restored money will likely go to existing infrastructure because canceled trials can't easily be restarted.

–JOCELYN KAISER

With reporting by Jennifer Couzin.

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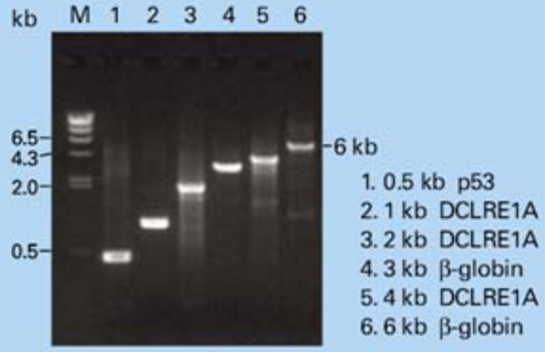

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SCIENTIFIC PUBLISHING

Osaka University Researchers Reject Demand to Retract *Science* Paper

Because of "numerous questions," Osaka University's Graduate School of Medicine has told one of its research groups to retract a 2004 *Science* paper on an insulin-mimicking protein secreted by fat tissue. The school's dean, Masaya Tohyama, last week held a press conference to issue the unusual demand, which came after a year-long investigation.

The school has not alleged scientific misconduct, and the paper's corresponding author, Iichiro Shimomura, says the issues raised by the investigation, such as ignoring data that complicated the paper's conclusions, do not warrant retraction. The metabolism researcher says he and the other authors are considering legal action against the university for how it handled the case.

The paper in question, published online in December 2004 and in print in the 21 January 2005 issue of *Science* (p. 426), concerns a protein dubbed visfatin. Shimomura and 21 colleagues at Osaka and three other Japanese companies or institutions reported that the protein is secreted by fat tissue and that its levels in blood increase during the development of obesity. Visfatin also exhibited insulinlike effects in cultured cells and lowered plasma glucose levels when administered to mice. The authors concluded that further studies of visfatin may lead to "new therapies for metabolic disorders like diabetes." The publication has been cited in some 60 papers, as indicated by *Science*'s online tracking system.

According to a statement released by the medical school, its committee for research integrity set up an investigating subcommittee in June 2006 in response to allegations of impropriety. The subcommittee's recommendation to retract the paper was endorsed by the school's faculty council on 14 June. The council decided, however, not to discipline the researchers. "There was no clear indication of any fabrication of data," says Tohyama. The dean says that the school does not intend to make the investigating committee's report public.

One concern, according to Shimomura, was that certain data were not included in the paper. The team had tried to create male and female heterozygous knockout mice, animals in which one of the two copies of the visfatin gene is disabled. Shimomura says that in such knockout mice, the expression level of the targeted gene should be half that found in typical mice. However, for the female heterozygotes, the expression level was not lowered that much, leading the team to conclude that the mice were not adequate models. "We had a reason for not including the [female] data," Shimomura says.

Fat problem. A paper describing a protein secreted by fat cells (*above*) has been called into question.

Harvey Lodish, a biologist at the Whitehead Institute for Biomedical Research and the Massachusetts Institute of Technology, both in Cambridge, says that the decision to drop the female heterozygotes "seems all right to me." But Lodish, who co-authored a commentary in *Science* on the research, says that because of other questions about the work, "we reserved judgment as to the reality of visfatin as a secreted insulin-mimetic hormone."

Shimomura says the group responded to other issues raised by the investigating committee in a rebuttal and stands by its original results. In a statement issued by *Science*, its editor-in-chief, Donald Kennedy, said the journal was taking the matter "very seriously." "We were aware that an investigation of scientific misconduct was under way at the University of Osaka Medical School and have been in contact with the dean for a number of months," added Kennedy. "We have been notified that the investigation is complete but have not been informed of the university's final determination."

This is the second time Shimomura has been a corresponding author on a problematic paper. In November 2005, he retracted a paper published by *Nature Medicine* a year earlier when it was found that the first author, Nobuyasu Komazawa, had fabricated data. Komazawa was not a contributor to the visfatin paper. **–DENNIS NORMILE**

CREDIT: FRED HOSSLER/VISUALS UNLIMITED/GETTY IMAGES

NIH Mapmakers Stalk Terra Incognita

Two hot biological research areas—epigenetics and the microbes our bodies host—will lead Roadmap 1.5, the second round of research initiatives that cut across all 27 institutes and centers at the National Institutes of Health (NIH). Alan Krensky, incoming director of NIH's new planning office, says solicitations for these two 5-year programs will go out this fall. Epigenetics will catalog genetic changes that affect gene expression but don't involve a change in DNA sequence. The Human Microbiome Project will examine the body's microbial communities and their relation to disease. Two more projects to start as pilots include work on human phenotyping and protein probes. NIH projects spending $30 million next year and $80 million for each of the next 4 years. **–JOCELYN KAISER**

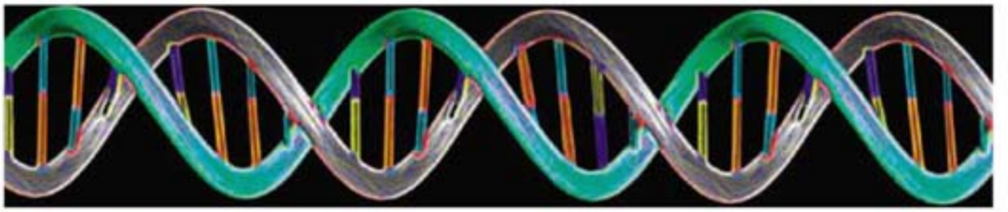
Patent Office Goes MySpace

The U.S. Patent and Trademark Office has begun a pilot program (*Science*, 19 May 2006, p. 982) to solicit outside input on computer technology patent applications. IBM, Intel, General Electric, Red Hat, and Hewlett-Packard have each given permission for one patent application to receive public scrutiny, and some 550 lawyers, programmers, and laypeople have signed up to participate. The patent office is hoping that the additional reviewers will help it spot proposed inventions that are not new. **–ELI KINTISCH**

Settlement Reached in Data Case

Leaders of the world's largest genetic biobank for children's health research say they're in the clear after settling a big lawsuit filed by deCODE Genetics in Reykjavik, Iceland.

The Children's Hospital of Philadelphia (CHOP) disclosed last week that it has paid an undisclosed sum to deCODE to end a suit in which deCODE accused several former employees of plotting to "steal" deCODE's computer programs and data (*Science*, 6 October 2006, p. 30). CHOP denied the allegations and countersued. In the settlement, deCODE and CHOP agree to withdraw allegations, and CHOP promises not to use deCODE's proprietary material. Philip Johnson, CHOP's scientific director, says that the hospital never used, nor intended to use, anything from deCODE, and that several research papers now in press are proof of progress despite the dispute. In a statement, deCODE's chair, Kári Stefánsson, says he's pleased to have reached an agreement with "a noble institution." **–ELIOT MARSHALL**



DNA SYNTHESIS

Gene-Synthesis Companies Join Forces to Self-Regulate

In 2004, Blue Heron Bio, a gene-synthesis company in Seattle, Washington, received a request by someone in the United States for a DNA sequence that would help a gene produce a toxin more effectively in an edible plant. Another order that year, by an organization in the Middle East, was for a part of the smallpox genome. Afraid that the DNA might fall into the hands of terrorists, the company declined both requests.

But it didn't have to. Existing laws in the United States, as well as most other countries, do not require companies to screen DNA orders, let alone turn down suspicious requests or report them to any government or body such as the United Nations.

Now, Blue Heron Bio and a host of other gene-synthesis companies have proposed guidelines for screening and handling the growing number of DNA orders. Founding members of the International Consortium for Polynucleotide Synthesis (ICPS) lay out the oversight framework in a commentary published this month in *Nature Biotechnology*. "We think this is a sensible way of handling the risks without slowing down the field," says co-author John Mulligan, chair of Blue Heron Bio.

Environmental activists are bristling at the proposal, which they say is an attempt by industry to preempt or minimize government regulation. The absence of formal oversight, they claim, makes the rapidly advancing field of synthetic biology a fertile ground for bioterrorism as well as ecologically catastrophic accidents. "It's not for a handful of scientists and entrepreneurs who have a vested interest in the technology to control the discourse or determine regulatory frameworks," says Hope Shand, research director of the ETC Group.

The topic is sure to provoke debate next week in Zurich at Synthetic Biology 3.0, the third in a series of meetings that brings scientists together to discuss technical, societal, and ethical issues related to the field. Even a co-author of the ICPS framework rejects its plea for governments to stay on the sidelines. Harvard University biologist George Church, a co-founder of Cambridge-based gene synthesis company Codon Devices, says he recommends government surveillance of companies, institutions, and researchers doing synthetic biology "since the stakes are so high."

At last year's Synthetic Biology 2.0, Church and other biologists publicly discussed security implications of their research, and some even raised the idea of a moratorium. But after a contentious debate, meeting participants issued only a vague call for self-regulation that infuriated the ETC Group and other watchdog groups (*Science*, 26 May 2006, p. 1116).

"We think this is a sensible way of handling the risks without slowing down the field."

—John Mulligan, Blue Heron Bio

"It's not for a handful of scientists and entrepreneurs who have a vested interest in the technology to control the discourse."

—Hope Shand, ETC Group

Under ICPS's proposal, customers placing orders would identify themselves and their home institutions and provide information about their capability to handle biological agents. Companies would be obligated to use industry-approved software to check orders against a list of potentially dangerous sequences and report problematic requests to government authorities. Founding members of the consortium say they already follow these steps.

So far, the push for self-regulation among synthetic biology researchers and companies has warded off government involvement. Neither the U.S. Department of Homeland Security nor any members of the U.S. Congress have called for binding legislation over the field. Some government officials have pointed out that the country's select-agent rules, which require individuals and institutions to register with the government in order to work with certain pathogens and toxins, already exert indirect pressure on gene-synthesis companies to screen orders.

The National Science Advisory Board for Biosecurity (NSABB), a panel appointed by the Department of Health and Human Services to minimize risks posed by "dual-use" life sciences research, has endorsed self-governance. In draft recommendations on synthetic biology unveiled earlier this year, the board suggested developing standards for screening DNA orders but didn't suggest new regulations (*Science*, 27 April, p. 529). If the government were to get tough in this area, "it could drive business overseas," says David Relman of Stanford University in Palo Alto, California, who heads NSABB's synthetic biology group.

Although praising the ICPS consortium for trying to address the risks posed by synthetic biology, some are troubled by the lack of regulatory teeth in the framework. Alan Pearson of the Center for Arms Control and Non-Proliferation in Washington, D.C., asks who would hold DNA-synthesis companies and their clients accountable if they ignored the guidelines. Pearson is surprised that the consortium did not recommend modifying the select-agent rules to cover requests for and manufacture of DNA sequences related to such agents.

The paper dismisses another obvious idea: a centralized government body that clears all DNA orders. That "impractical option," say the authors, would scare off clients concerned about breach of privacy. "Most of our customers are very concerned about protecting the confidentiality of the sequences they are ordering, which are often highly proprietary," says Mulligan.

Church stresses the need to develop better software to screen DNA requests. Industry representatives say the number of false alarms they have to look into makes the process tedious. "No matter what the regulations, good software is our first line of defense," says Church, adding that the consortium plans to pool resources and request government funding to improve screening.

Although Church contends that self-governance is not enough, his voice wasn't enough to persuade the ICPS that government regulation is needed or feasible. "I think it would take a significant change in the culture of science and business to support broad-scale surveillance of DNA sequences that people work on," says Mulligan. "I think it would be a difficult change to make."

–YUDHIJIT BHATTACHARJEE

CREDIT: JAMES J. CARAS/NATIONAL SCIENCE FOUNDATION

SCIENTIFIC PRIZES

U.S. National Medals: For Men Only?

Any day now, the Bush Administration will announce the eight winners of the 2006 National Medal of Science, touted as "the country's highest honor for scientific achievement." For the first time in 4 years, the honorees will include a woman. That's a terrible record, say advocates of greater diversity in science, and sends a disturbing message about who is capable of doing world-class science.

"I'm a female engineer, and I'm appalled," confesses Mayra Montrose, who manages the program at the National Science Foundation. "It's unbelievable."

The paucity of women also extends to the National Medal of Technology, a similarly prestigious award run by the Commerce Department. Only three women have been honored as individuals since the program began in 1983, and none since 1996. (Each class typically also features corporate winners, ranging from a team of scientists to the entire company. The 2005 winners announced earlier this month, for example, feature a female member of a group at Wyeth Pharmaceuticals that developed a children's pneumococcal vaccine.) It's certainly not a new phenomenon: Of the 425 science medalists since 1962, only 30—about 7%—are women. And there have never been more than two in a single year.

Advocacy groups aren't accusing the selection committees, which typically include several women, of bias. Instead, they point to a culture that undervalues the contributions of women, along with a tendency of women to be less aggressive in seeking such honors. The combination, they say, results in a trickle of nominations. That appears to be the case for the science medals. The pool of 188 candidates reviewed last year, for example, included 11 women—the same percentage as their historic rate of success.

The phenomenon also suggests an obvious solution: Get more women to apply. That's precisely the goal of Project RAISE (Recognition of the Achievements of Women In Science, Medicine, and Engineering), run by the Society for Women's Health Research and aimed at awards programs of all types. "There's a certain skill involved in the application process, and we need to help women learn how to do it well," says co-founder Stephanie Pincus, a former chair of the department of dermatology at the University of Buffalo, New York.

Pincus says she was shocked to learn how few nominations of either sex—about three to four dozen—are submitted each year to the two national medal selection committees. (Anyone can nominate anyone, including oneself, and a nomination remains active for 4 years.) The low number suggests that efforts such as RAISE can play an important role. "Boosting the number of applications won't solve the problem, but it's an important first step," she argues.

Members of the selection committees agree that something needs to happen. "It's definitely an issue for the committee," says Linda Katehi, provost of the University of Illinois, Urbana-Champaign, and a member of the technology medals panel. "We have people from diverse backgrounds in science, and we need to find a way to recognize them."

–JEFFREY MERVIS

CREDIT (TOP): THE NATIONAL MEDAL OF SCIENCE

Science Editor-in-Chief to Retire

Donald Kennedy has announced that he will be stepping down as *Science*'s editor-in-chief. Kennedy, who has been the magazine's top editor since 1 June 2000, has told the Board of Directors of AAAS, *Science*'s publisher, that he plans to retire around the end of the year but will continue as editor-in-chief until a successor is found. A search committee chaired by AAAS President David Baltimore is being established to conduct an international search. "Don Kennedy has provided superb leadership to *Science*, and it will be very hard to find a successor," says AAAS CEO Alan Leshner. "He's set the bar very high."

Report Backs Interspecies Lines

A report by Britain's Academy of Medical Sciences has bolstered the political case for allowing the creation of so-called cybrids, in which human DNA is inserted into animal eggs to generate human embryonic stem cells. The U.K. Human Fertilisation and Embryology Authority (HFEA) is currently conducting a public consultation on the practice, with a fall decision expected.

The report asserts that research involving interspecies embryos raises "no substantive ethical or moral reasons not to proceed" provided that the usual regulations are followed, panel chair Martin Bobrow of the University of Cambridge said in a statement. Most nations, including the United Kingdom, require that no human research embryos be allowed to survive beyond 14 days.

Cybrids could allow nuclear transfer without tapping the limited supply of human eggs. The issue came to a head last year when two groups applied to HFEA for permission to work with cow eggs instead. Critics find distasteful the idea of mixing animal and human material, and the practice has been banned in Australia and several other nations. The British government has proposed legislation that would ban the practice but allow exceptions.

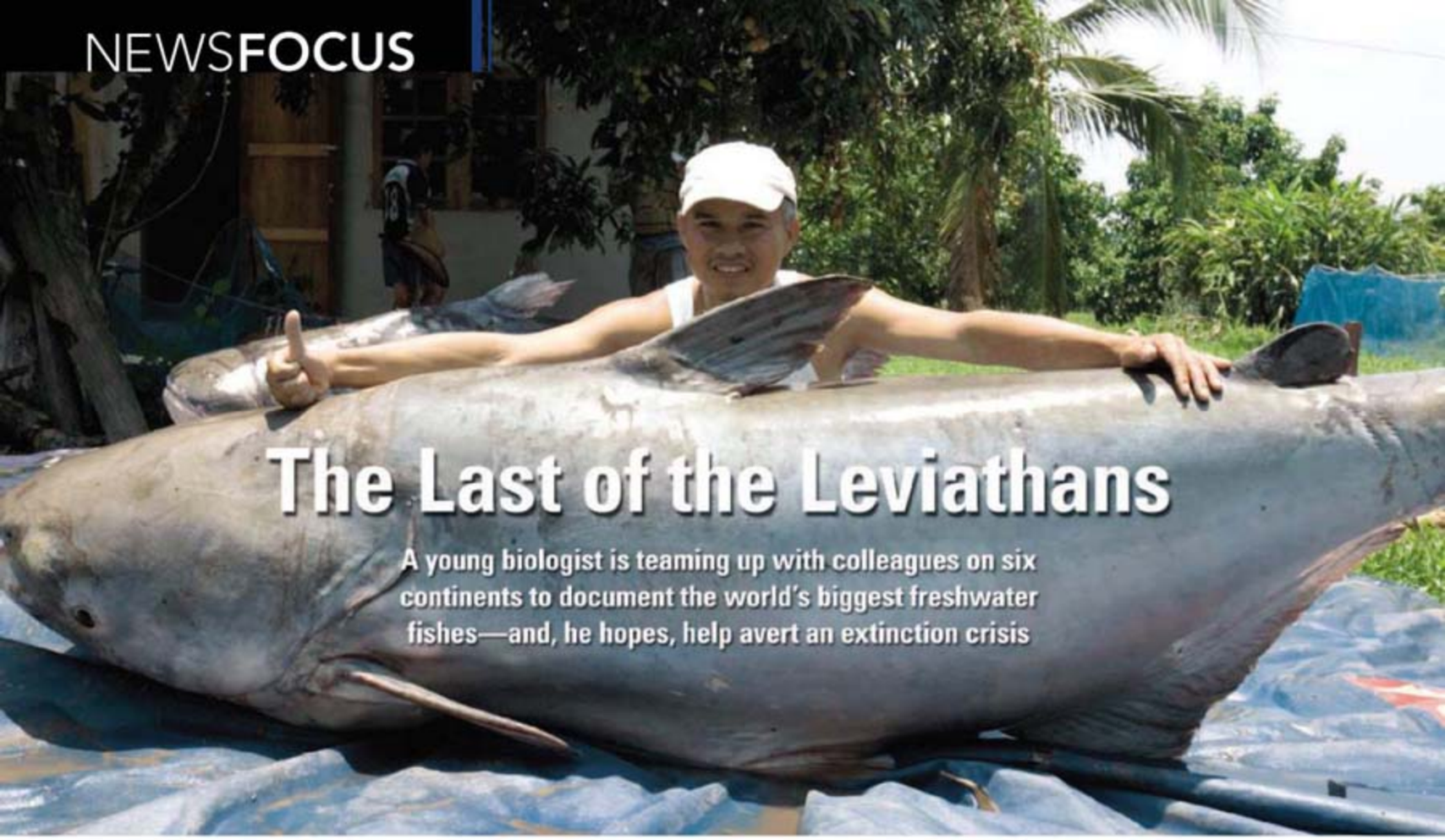
–CONSTANCE HOLDEN

What's That Smell?

The Environmental Protection Agency (EPA) last week launched the first national study of air pollutants from dairy cow, swine, and poultry farms. For 2.5 years, researchers from eight universities will measure emissions of hydrogen sulfide, ammonia, and other gases from livestock farms in nine states across the country. EPA will then create a model to enable individual operations to check whether they need an agency permit.

Environmentalists say the study is not comprehensive enough and may underestimate emissions. EPA has the authority to order farms to do their own monitoring, but it chose instead to study just 24 sites as part of a 2001 deal with industry. In exchange for not being regulated, more than 2600 operations with 14,000 farms agreed to contribute a total of $14.6 million for the study. "Without a better study, I'm not sure this will lead to anything but more lawsuits and delay," says Karla Raettig of the Environmental Integrity Project in Washington, D.C. The D.C. Court of Appeals heard arguments this year on a case activists brought challenging EPA's voluntary approach. It's unclear how that case, which is pending, could affect the research.

–ERIK STOKSTAD



The Last of the Leviathans

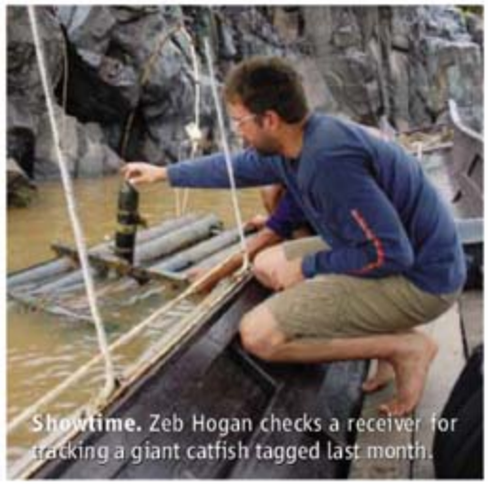
A young biologist is teaming up with colleagues on six continents to document the world's biggest freshwater fishes—and, he hopes, help avert an extinction crisis

CHIANG KHONG, THAILAND—Eddies and whirlpools, weak and evanescent, swirl water the color of milk chocolate in a narrow stretch of the Mekong River between Laos and Thailand. Zeb Hogan asks the longboat driver to steer toward a rocky island. As slate-gray thunderclouds bear down from the north, the boat eases alongside a small bamboo raft roped to shore in a tiny cove.

Hogan leans out and flips the raft over. Fixed underneath is a metal cylinder that holds an acoustic receiver. Hogan, a fisheries biologist with the University of Nevada, Reno, is checking to see if this and 16 other receivers along a 400-kilometer stretch of the river are ready for showtime. On 6 May, near Chiang Khong, he stuck an acoustic transmitter on the dorsal fin of a Mekong giant catfish. It was the only one caught this year, and the first ever tagged successfully, in Thailand. The receivers will track its movements. "We think this part of the Mekong is critical habitat," Hogan says. "Somewhere around here is the spawning area."

But even after a decade of reconnaissance in the Golden Triangle, the highlands where Laos, Myanmar, and Thailand meet, Hogan is not quite sure where that area is. The only certainty is that the giant catfish (*Pangasianodon gigas*), one of the world's biggest freshwater fishes, is getting harder to catch. Hogan has a finger on the fading pulse of other Mekong monsters as well, including the giant pangasius (*Pangasius sanitwongsei*) and the giant freshwater stingray (*Himantura chaophraya*), both of which rival the giant catfish in sheer heft and which are also at risk.

Backed by the National Geographic Society (NGS), Hogan this spring has embarked on a 3-year "Megafishes Project" to document and protect the titans of the world's rivers and lakes: two-dozen-odd freshwater fishes that can top 200 pounds or 6 feet long (91 kilograms or 183 centimeters; see table, p. 1686). Many of these sumo-sized species are on the ropes, pummeled by overfishing and habitat degradation. Hogan's quest has begun on the Mekong, whose 1200-plus fish species make it the world's most biologically diverse river basin of this size. The Mekong is in the grip of a global calamity unfolding in fresh water, which accounts for 0.01% of all the planet's water but is home to at least 10,000, or about 40%, of known fish species. "Everywhere we look, the largest fish are disappearing," Hogan says. "These are iconic fish," adds environmental scientist Thomas Lovejoy, president of the Heinz Center in Washington, D.C. "Much like tigers on land, they are flagship species representing the wonders of life in rivers."



Showtime. Zeb Hogan checks a receiver for tracking a giant catfish tagged last month.

Lovejoy and others laud Hogan for sounding the alarm. The "freshwater extinction crisis" deserves more attention, says Julian Olden, an aquatic ecologist at the University of Washington (UW), Seattle. Megafishes, adds Peter McIntyre, a fish biologist at Wright State University in Dayton, Ohio, "are emblematic of the problems of overexploitation and habitat alteration facing freshwater fishes around the world." By stirring up interest in the megafishes, Hogan "is likely to benefit numerous other species," McIntyre says.

Large freshwater fish "are uniquely vulnerable," says David Dudgeon, an aquatic ecologist at the University of Hong Kong. They can live for decades, and "an awful lot of bad things can happen before they mature," he says. The consequences are particularly devastating for species confined to a single river. "If you screw up that habitat, they're gone," Dudgeon says.

CREDITS (TOP TO BOTTOM): SUTHEP KRITSANAVARIN; COURTESY OF ZEB HOGAN

Moby Dick of the Mekong. Caught in 2005, this record giant catfish weighed 293 kilograms.

Unlike pandas, their cuddly appeal is nil. "People have a hard time sympathizing with fish," acknowledges Hogan. But when it comes to freshwater creatures, these "are the largest ones out there, and they're in big trouble."

Trophy fish evangelism

Last year, when a lengthy search for the baiji, or Yangtze River dolphin, came up empty, Hogan viewed the mammal's disappearance as an ominous portent. The Chinese team had anticipated a diminished population, not a total wipeout (*Science*, 22 December 2006, p. 1860). Similarly, Hogan fears that the Mekong River's giant pangasius is well on the way to oblivion. Unlike the giant catfish—a bottom feeder—the giant pangasius, also called the dog-eating catfish, is a predator. (Fishers snared it years ago by baiting hooks with dog flesh.) "The most common reaction I get is surprise that these species exist," says Hogan. By the end of World War II, pangasius specimens larger than 2 meters were rare in Thailand, and it may be extirpated from one of its original haunts, Thailand's Chao Phraya River. Now it's fighting a losing battle in the Mekong. "People aren't catching big ones anymore," Hogan says. The giant pangasius might disappear before basic sleuthing can be done, he says: "We know almost nothing about it."

Information is scarce—and time is running out—for most species on the megafish list. Take the arapaima, or pirarucu, a South American fish that must surface every 15 minutes or so to gulp air. The biggest arapaima (*Arapaima gigas*) topped 3 meters and 200 kilograms at one time. But their need to breathe makes them easy to harpoon. In recent decades, the average capture size has "drastically decreased," says arapaima expert Patricia Pinho of the University of California, Davis.

Like the giant pangasius, certain arapaima varieties may vanish before scientists become acquainted with them. Some species have not yet been described, whereas others have not been seen since the 1800s, says Donald Stewart, a fish biologist at the State University of New York (SUNY) College of Environmental Science and Forestry in Syracuse. He and his graduate students are studying arapaima in Brazil and Guyana. "Insufficient knowledge of the taxonomy and ecology" impedes conservation, says Stewart. "The first, last, and only meaningful analysis of species-level taxonomy for arapaima was in 1847!"

CREDIT: COURTESY OF ZEB HOGAN

The geography of human development could doom the megafishes. "In contrast to big sea dwellers, these riverine giants often live in close contact with dense human populations," says Stewart. In the Mekong, Amazon, and other river basins in the developing world, fishery interests usually trump preservation.

Dams, industrial effluents, and commercial navigation add to the pressure. "Freshwater systems get it every which way. It's hard to find a big river that hasn't been massively modified," says conservation ecologist Stuart Pimm of Duke University in Durham, North Carolina. An extreme case is the Yangtze, where a triple whammy of habitat modification, pollution, and heavy boat traffic may have turned the Chinese paddlefish (*Psephurus gladius*) into "the living dead," in that although individuals may still ply the river, the species itself could be doomed, says Dudgeon. The fish may be past the point of no return, he explains: Not even habitat preservation might save it now. "I've been searching since 2003 in almost all the Yangtze and have found none," says Wei Qiwei of the Yangtze River Fisheries Research Institute in Jingzhou.

Because many megafishes are migratory, "large dams are a huge threat," says Ian Baird, a geographer at the University of British Columbia (UBC) in Vancouver who works on the Mekong. Scientists and activists are up in arms over the Laotian government's plans to build the first hydroelectric dam on the Mekong south of China, at Siphandone near the Laos-Cambodia border. The dam would block a channel that migratory fish, such as giant catfish, use to bypass the Khone Falls, says Roger Mollot, a fisheries expert with the World Wide Fund for Nature (WWF) in Vientiane, Laos. That prospect, he says, "is obviously a major concern for fish biodiversity and fishing livelihoods."

This is not the sole menace in one of the most productive fisheries in the world. The Mekong Navigation Improvement Project intends to dynamite and dredge a stretch of the river north of Chiang Khong. "They're considering blasting in the presumed spawning grounds of the giant catfish," says Hogan. Dudgeon feels that blasting there "could be the last nail in the coffin for the species." Fortuitously, the work has been postponed out of national security concerns, explains Mollot: "The Thai government worries that blasting will alter the hydrology of the river," which, in that stretch, forms part of the Thai-Laos border.

Lassoed. This giant freshwater stingray measured 2 meters in width and 4 meters from stem to stern; fishers claim to have seen stingrays twice that size.

Thanks to quirks of topography or, rarely, sound management, a few megafishes are holding their own. Although subjected to intensive fishing, the giant perch (*Lates angustifrons*) in central Africa's Lake Tanganyika is lucky "in that it has a huge, deep lake to hide in and relatively low-technology fisheries methods to contend with," says McIntyre. "Their populations may well persist in the lake's depths despite the heavy fishing pressure." And in North America, the lake sturgeon (*Acipenser fulvescens*), decimated by anglers early in the 20th century, has rebounded. "They are relatively well-managed, and their populations remain stable," says Jake Vander Zanden, an aquatic ecologist at the University of Wisconsin, Madison. "There are important lessons in the success stories."

THE MEGA 20: THE WORLD'S LARGEST FRESHWATER FISH *


Giant barb


Lake sturgeon


Taimen


Giant stingray

COMMON NAME	SCIENTIFIC NAME	MAXIMUM SIZE	FOCAL RIVER SYSTEM	IUCN RED LIST (2006) CATEGORY	MAJOR THREATS†
Chinese paddlefish	*Psephurus gladius*	700 cm, 500 kg	Yangtze River Basin	Critically endangered	Harvest, habitat loss
Giant freshwater stingray	*Himantura chaophraya*	500 cm, 600 kg	Mekong River Basin	Vulnerable	Harvest, pollution
Wels catfish	*Silurus glanis*	500 cm, 306 kg	Widespread in Europe and Asia	Least concern	NA
Arapaima (pirarucu; paiche)	*Arapaima gigas*	450 cm, 200 kg	Amazon River Basin	Data deficient	Harvest
Soldatov's catfish	*Silurus soldatovi*	400 cm	Amur River Basin	Not evaluated	Harvest, habitat, pollution
Piraíba (laulau; lechero)	*Brachyplatystoma filamentosum*	360 cm, 200 kg	Amazon River Basin	Not evaluated	Harvest
Alligator gar	*Atractosteus spatula*	305 cm, 137 kg	Mississippi River Basin	Not evaluated	Unknown
Mekong giant catfish	*Pangasianodon gigas*	300 cm, 300 kg	Mekong River Basin	Critically endangered	Harvest, habitat loss
Giant barb	*Catlocarpio siamensis*	300 cm, 300 kg	Mekong River Basin	Not evaluated	Harvest, habitat loss
Giant pangasius (dog-eating catfish)	*Pangasius sanitwongsei*	300 cm, 300 kg	Mekong River Basin	Data deficient	Harvest, habitat loss, pollution
Putitor mahseer	*Tor putitora*	275 cm	Brahmaputra River Basin	Not evaluated	Harvest, habitat loss
Lake sturgeon	*Acipenser fulvescens*	274 cm, 125 kg	St. Lawrence, Great Lakes	Not evaluated	Harvest, habitat loss, pollution
Wallago (giant sheatfish)	*Wallago attu*	240 cm	Mekong River Basin	Not evaluated	Harvest
Mangar	*Barbus esocinus*	230 cm, 136 kg	Tigris River Basin	Not evaluated	Unknown
Mississippi paddlefish	*Polyodon spathula*	221 cm (including paddle)	Mississippi River Basin	Vulnerable	Harvest, habitat loss, pollution
Nile perch	*Lates niloticus*	200 cm, 200 kg	Nile River Basin	Not evaluated	Harvest
Pallid sturgeon	*Scaphirhynchus albus*	200 cm, 130 kg	Mississippi River Basin	Endangered	Harvest, habitat loss
Murray cod	*Maccullochella peelii peelii*	200 cm, 113.5 kg	Murray River Basin	Not evaluated‡	Harvest, habitat loss
Tanganyika lates (giant perch)	*Lates angustifrons*	200 cm, 100 kg	Lake Tanganyika	Endangered	Harvest
Taimen	*Hucho taimen*	200 cm, 100 kg	Selenge River Basin, Lake Baikal, Amur River Basin	Not evaluated	Harvest, habitat loss, pollution

*Excluding sturgeon species that move between freshwater and saltwater. †Threats have been divided into four categories: habitat loss/degradation (associated with agricultural land use, natural resource extraction, and human infrastructure, especially dams); harvesting (for food, medicine, fuel, or materials); invasive species (associated with competition, predation, hybridization, or pathogens/parasites); and pollution (atmosphere, land, or water). ‡Vulnerable in Australia.

CREDITS (TOP TO BOTTOM): COURTESY OF ZEB HOGAN; JEAN-FRANÇOIS HELIAS; SOURCES: ZEB HOGAN; DONALD STEWART; LEANDRO CASTELLO; DAVID DUDGEON; WEI QIWEI; IUCN

In many regions, however, conservation and science are far down the list of priorities. "There hasn't been a lot of emphasis on research," says Hogan. "There just aren't that many people doing this kind of work." And environmental protection is a novel concept in the Mekong River Basin. "Frankly, most of us are only beginning to learn about conservation," says Uthairat Na-Nakorn, a fish geneticist at Kasetsart University in Bangkok. In Thailand, she says, "when an outsider raises an issue, the issue becomes more important."

That's where Hogan is making a mark.

Hogan, 33, says his megafish epiphany came 10 years ago, when he spent the 1996–97 academic year on a Fulbright student fellowship at Thailand's Chiang Mai University. "I came to Thailand at a very good time," he says. Interest in Mekong ecology was waxing as a result of a major study into how future dams could harm the lower Mekong. After learning Thai, Hogan visited village markets to record the kinds of fish for sale. He narrowed his focus to the pangasiids, a group of migratory Mekong catfishes that spawn in spring, at the start of the rainy season. Hogan and colleagues determined that the silver-toned catfish (*P. krempfi*), unlike its cousins, is anadromous: It's a saltwater species that enters the river only to spawn. They nailed this from the high strontium levels in the catfish's otoliths, or ear stones, and an isotopic signature in muscle tissue indicative of growth in a marine environment, the South China Sea.

Hogan says his seduction by the Mekong giant catfish "happened by accident," after he had begun doctoral ecology studies at the University of California, Davis. In April 2001, he went to Chiang Khong for the annual hunt of *pla beuk*, or buffalo fish, as the giant catfish is called in Thailand. A century ago, fishers hauled in hundreds per year. By the time Hogan arrived on the scene, he says, "it was the end of the heyday."

Fishers in the Chiang Khong area had landed 20 in 1999. Hogan hung around Chiang Khong for 1 month in 2001, but no giant catfish were netted. The same thing—nothing—happened in 2002 and 2003. "There was a feeling, 'Hold on, we may have a problem here,' " Hogan says. There was a small rebound in 2004 and 2005, when seven and four, respectively, were caught. Then only one was netted by Laotian fishers last year, and the one this year that was tagged. Several giant catfish each year continue to be captured, and usually released, in the Tonlé Sap region of Cambodia, where the fish rears its young after spawning. The statistics are grim, and the ecological forces behind the year-to-year fluctuations upstream are a mystery. "No one really knows what's happening in the river," Hogan says.

Goliaths of the Amazon. Protecting megafishes will require more research and in some cases litigation, says Donald Stewart, seen here with arapaima harvested by fishers in Brazil's Mamirauá Reserve.

CREDIT: L. CASTELLO

Big-game hunting

On the beach on the Laotian side of the Mekong, across from Chiang Khong, several Thai fishers are lounging beneath a roof thatched with palm leaves. Their fraternity holds exclusive fishing rights for the giant catfish. In Thailand, only residents of Hat Khrai, a village next to Chiang Khong, can claim that honor, which has passed from father to son for generations. In recent years, the season has been limited to 1 week per year.

This May, after a voluntary moratorium in 2006, members of the Hat Khrai Mekong Giant Catfish Club are anticipating a return to the hunt. The thick-twine gill nets, deployed exclusively for the giant catfish, are laid neatly in a few of the longboats tied to shore. The fishers, languid in the midday heat and eyes rheumy from homebrewed rice whiskey, are planning to hit the river in the evening. A short walk down the beach, a few of their sons are sprawled out under a tarp. The rainy season descended a month early, and with a squall approaching, the young men are about to get drenched. "The catfish are coming," one says, prophetically. He's worth heeding: In 2005, he and two friends landed the world-record giant catfish, a 2.7-meter-long, grizzly bear–sized titan that weighed 293 kilograms. With catches since then so rare, that specimen may well have been the last of the leviathans.

This season, the fishing is strictly for scientific purposes; any giant catfish snared must be tagged and released in return for cash. For the giant catfish tagged on 6 May, Hogan and a documentary film crew forked over the equivalent of $1500, roughly market value. The meat, a delicacy in Thailand, fetches up to $15 per kilogram. "Thai people think that eating it will give them good luck forever," says Sujin Nukwan, director of the Inland Aquaculture Research Institute in Ayutthaya, Thailand. Hogan tried it a few years ago. "It tastes muddy," he says.

The older fishers are an invaluable historical resource for Hogan. So far, he has interviewed 60 fishers over the age of 40, asking them, among other things, to compare average catch and size now compared with 20 years ago—standard World Conservation Union criteria for assessing fish stocks. The fishers also lend a hand with tracking tagged fish. "For each receiver, there's a fisherman responsible," Hogan says. "Our ultimate goal is to nail down where the Mekong giant catfish is spawning."

After finishing his work at Chiang Khong, Hogan traveled down the Mekong to Cambodia, film crew in tow, to look for a giant stingray, which reportedly can reach 500 kilograms or more. "Cambodia is the last refuge for some of these species," he says. Fishers told him stories of "absolutely enormous" specimens. One sketched a stingray in the sand that measured 4 meters wide and twice as long. "He had a tale to match; think sinking boats à la giant squid," Hogan says. But Hogan came away empty-handed.

Letting the cat out of the bag

The plight of the megafishes is beginning to draw more international experts to the area. At a WWF-sponsored meeting on the Mekong giant catfish in Vientiane last month, freshwater scientists and policymakers mulled a fishing ban or limited catch for scientific studies. "We concluded that there is a lack of information to base sound decisions," says WWF's Mollot. Major knowledge gaps include exactly how many giant catfish are caught each year in the Mekong River Basin, where the fish spawn, and whether giant catfish in Cambodian waters in the lower Mekong and fish in the upper reaches of the river are a single population.

Officials are leaning toward permitting a scientific catch—including taking fin tissue samples for genetic analyses—to get at these questions. "We need rigorous research," says Mollot. At the Vientiane meeting, Lao and Thai officials agreed to forge a common policy for managing the giant catfish. WWF, Mollot says, will also seek to get the species' future "on the table" when policymakers discuss development of the Mekong River Basin.

They might look to Brazil for guidance. In the Silves region of the Central Brazilian Amazon, indigenous people have set up a zoning system to protect floodplain lakes that are critical to the arapaima and a second imperiled fish, the tambaqui (*Colossoma macropomum*). Three management regimes are now in place: "sanctuary lakes," nursery grounds where fishing is forbidden; "maintenance lakes," in which only local people can fish, and "open-access lakes." In Lake Purema, declared a sanctuary in the 1980s, Pinho has observed that arapaima are doing better: The average body size of individuals, and the population, are both growing. She is now working with the indigenous people to establish federally protected reserves throughout the country.

Whether such measures will succeed in the long run will depend largely on community acceptance—and funding. "The problem is that there just isn't much money for protecting these species, or for international fisheries research in general," says Hogan. The Mekong Wetlands Biodiversity Conservation and Sustainable Use Programme kicked in $50,000 for receivers and tags for Hogan's effort before the program was killed last year and its budget redirected for climate change research. NGS is chipping in $35,000 a year for equipment.

As *Science* went to press, Hogan, an NGS "emerging explorer," and his Megafishes Project co-director, limnologist Sudeep Chandra of the University of Nevada, Reno, were in Mongolia. There they hope to map critical habitat of the taimen (*Hucho taimen*), the world's biggest salmon, and ensure that its catch-and-release fishing season is timed to open after spawning. Then Hogan is off to the Amazon—for him, aqua nova—to study the arapaima. "The idea is not to go in and take over arapaima research," he says; it's to get a snapshot of the fish's conservation status. "Zeb tries to involve local scientists," says UBC's Baird. Other megafishes on other continents await Hogan's attention in 2008 and 2009. At the same time, UW's Olden cautions not to lose sight of the smaller picture. "We need to recognize that both the giant and tiny fishes of the world are at risk of global extinction," he says.

With the threat of extinction growing by the day for some megafishes, Hogan has redoubled his efforts to get the looming catastrophe to resonate with the public. The toughest crowd to reach may be the policymakers who are best positioned to protect the fishes. "There's slow movement forward," says Hogan, who last year was appointed scientific councilor for fish for the U.N. Convention on Migratory Species. "There's not a lot of concern, but there's more concern than there ever has been," he says. This is only the first step on a long road, cautions SUNY's Stewart. "Real solutions will only come from many years of scientific investigation, education, negotiation, legislation, and maybe, in some cases, litigation," he says.

Some experts say that the battle to save giant fish will be won or lost in the Mekong. "The Mekong represents our last, best chance," argues Dudgeon. Pollution levels on the river are not horrendous, he says, and the lower Mekong still mostly follows its natural flow. Moreover, millions of people depend on the river. Fishery resources "are a part of the cultural fabric of rural life in the Mekong Basin," notes Mollot. Thus for the Mekong giant catfish and other freshwater whoppers, Hogan's success at translating concern into action may mean the difference between a resurgence in the wild and a gloomy existence as the last living representatives of their species.

–RICHARD STONE

CREDIT: COURTESY OF ZEB HOGAN

ON LIFE SUPPORT

AYUTTHAYA, THAILAND—Under a blazing sun, a technician in a straw hat lobs a softball-sized, mushy tan mass into a circular concrete pool. Five Mekong giant catfish, each more than a meter long and weighing 50 to 60 kilograms, glide listlessly past the food offering. They aren't interested 2 hours before their scheduled daily feeding here at the Inland Aquaculture Research Institute in Ayutthaya, the medieval capital of Siam.

Whether due to dull appetite or small enclosure, these catfish are bantamweights compared with some of their wild cousins. And no one knows whether hatchery-bred giant catfish can even cut it in the wild. "The fear is that they will not understand the environmental cues to begin a spawning run," says Roger Mollot, a fisheries expert with the World Wide Fund for Nature in Vientiane, Laos.

Last year, a team led by fisheries biologist Zeb Hogan of the University of Nevada, Reno, tagged 40 fish of several species, including 18 captive-bred giant catfish released into the Mekong south of Chiang Khong, just before spawning season. Wild catfish swam upstream, as expected. The captives drifted down with the current. "The take-home lesson is that the hatchery fish are not adapting to the river," Hogan says.

But captive breeding of the Mekong giant catfish does offer one benefit: It eases fishing pressure on wild stocks. In a tank near their full-grown cousins at the Ayutthaya institute, several dozen younger giant catfish about 10 centimeters long are thrashing about, displaying much more energy than the adults. They're about to be sold for the equivalent of $1 apiece to a farmer who will raise them in a reservoir. Thailand's 25-year-old program to breed the Mekong giant catfish has matured into a sustainable aquaculture industry, Hogan says. But captive breeding, he and others say, would only offer a last-gasp option for conservation. **–R.S.**

In transit. New rules will allow bald eagles to be moved from airports and other dangerous locations.

SPECIES CONSERVATION

Can the Bald Eagle Still Soar After It Is Delisted?

Scientists hope that new rules will ensure the survival of a national symbol once it's dropped from the endangered species list

Just 40 years ago, the bald eagle seemed headed for extinction in the conterminous United States. Nesting females were accidentally crushing their eggs, which were weakened by the ubiquitous insecticide DDT. Populations spiraled downward. By 1963, only 417 pairs were still raising young in the lower 48 states.

But the national icon began to bounce back after Congress banned DDT in 1972 and passed the Endangered Species Act (ESA) in 1973. Last year, there were nearly 10,000 successful breeding pairs. "It's one of the greatest wildlife success stories in the history of this country," says attorney John Kostyack of the National Wildlife Federation in Reston, Virginia.

Now, the U.S. Fish and Wildlife Service (FWS) in Washington, D.C., is poised to declare victory for the majestic avian by removing the bald eagle from its list of threatened species by a court-ordered deadline of 29 June. Bald-eagle experts agree with the move, although some argue that a small population in the Southwest isn't ready for delisting. Many remain worried, however, about how well new rules to protect the birds will be enforced and the extent to which populations will be monitored. They also call for safeguards to prevent development from encroaching on the birds' remaining habitat.

"Eagles will be a test case," says conservation biologist Bryan Watts of the College of William and Mary in Williamsburg, Virginia. What happens once they are delisted, says Watts, "will say a lot about how our culture will handle other conflicts between landowners and species."

Act two

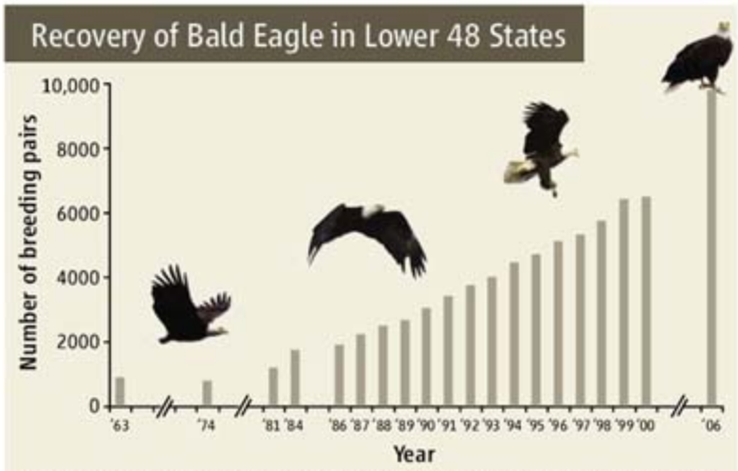
FWS, which is legally responsible for making sure species don't falter once they are delisted, first proposed removing the bald eagle from the endangered species list in 1999. ESA considers a species to be fully recovered when the threat has been reduced, extinction is unlikely, and there is little chance of the species becoming endangered again "within the foreseeable future." To meet those goals, FWS created recovery plans for different regions of the country that spelled out specific targets and approaches to achieve them. By 1999, eagle populations exceeded the goals. In 2005, the Pacific Legal Foundation (PLF) in Sacramento, California, sued FWS for not completing the delisting process on time. A U.S. district judge agreed and ordered the agency to finalize its listing proposal.

FWS staff have spent years trying to smooth the transition from the ESA to a 1940 law, the Bald and Golden Eagle Protection Act (BGEPA), that was designed to prevent people from shooting eagles and has remained on the books. On 1 June, FWS proposed new regulations under the old law intended to prevent any activity "likely to cause, based on the best scientific information available," harm to the birds. "That [language] was a sigh of relief," says James Bednarz, a conservation biologist at Arkansas State University, Jonesboro. It replaced earlier language that biologists worried would have prevented enforcement officers from acting until after damage had been done.

The new rules also make the BGEPA more flexible than it used to be. For example, wildlife managers would be permitted to remove nests near airports and transport eagles to safer locations. As with the ESA, landowners will be able to apply for permits for activities likely to affect the birds, such as building a road near a nest. Permission will be granted if there is no practical alternative and the landowner proposes some way to mitigate the potential harm by, say, not building the road during nesting season. The agency will accept public comments on the rules until 4 September. This month, PLF said it may challenge the new definition of "disturb" as too broad and that it would make the BGEPA the functional equivalent of the ESA.

Although biologists agree that there are healthy populations of bald eagles in most of the country, one exception may be the Southwest. The population there has grown, from three breeding pairs in 1971 to 43 in 2006, but some scientists don't think it is large enough to remain stable. Last year, a panel of scientists assembled by the Raptor Research Foundation, a scientific society, concluded that development still poses a significant threat and that the population is vulnerable because of low productivity and high mortality. "They're not ready to be delisted yet," says Steven Sheffield, a wildlife biologist at Bowie State University in Maryland, who chaired the panel.

But most biologists contend that delisting is the right thing to do even in the Southwest. Grainger



Upswing. Banning DDT has helped the national population of breeding bald eagles to grow. The data come from state surveys, which are not all done every year.

CREDITS (TOP TO BOTTOM): AP; JUPITER IMAGES; SOURCE: FWS

Hunt of the University of California, Santa Cruz, who studied the bald eagle population in the 1990s, says the Arizona Game and Fish Department—which signed a multiagency conservation agreement in January to prevent a decline of the species—does a good job of conserving the existing habitat: "If this population does start having problems, you would see it coming and could make regulatory changes."

The Center for Biological Diversity (CBD), an advocacy group in Tucson, Arizona, doesn't want to take any chances, however. The group petitioned FWS last year not to delist the Southwest population and sued after the agency did not respond by its own 90-day deadline. The case is pending before the U.S. District Court in Arizona, and Kieran Suckling of CBD says he may ask the court to put the delisting on hold nationwide until it resolves the matter.

After delisting

Once populations are delisted, attention will shift to how well the new rules are enforced. A major concern is that delisting will give some landowners the erroneous impression that they can disturb eagles, Watts says. "We get calls all the time from developers waiting for delisting because they think protections will be reduced a great deal." Watts believes that stiffer enforcement after delisting will be needed to convey the message that the eagle is still protected and that penalties apply.

Watts and other biologists worry that states, which traditionally do most of the monitoring, will slack off once the eagle is delisted. Monitoring will be even more important then, says Watts, to shed light on how a species is impacted by delisting. "It's going to be the most interesting time in 20 years," he says. FWS officials admit they will spend fewer dollars on eagles once they are delisted, and many states are likely to follow their lead. That's not necessarily a bad thing, researchers admit, if it frees up resources to help other endangered species in dire straits.

Ultimately, the fate of the bald eagle is likely to rest on the amount of suitable habitat. That puts the onus on state and local governments to control development as best they can, and on citizens to manage their land with eagles in mind. But that may be a tall order. For example, real estate values along the Chesapeake Bay, which is home to a large population of eagles, have skyrocketed, increasing pressure to develop land. The eagles, Watts predicts, "won't recover from suburban sprawl like they did from DDT."

–ERIK STOKSTAD

Genetic historians. Researchers have started to use large-scale genome data from people worldwide to learn about human evolution.

CREDIT: JUPITER IMAGES

POPULATION GENETICS

Population Geneticists Move Beyond the Single Gene

Genetic information about large numbers of individuals is a population geneticist's dream come true. But it can also be a challenge

For much of his 14-year career as a population geneticist, Lluís Quintana-Murci of the Pasteur Institute in Paris, France, has focused on one or two genes at a time, trying to understand their history during human evolution as well as any roles in preventing or causing disease today. That so-called candidate gene approach has worked well, within limits.

For example, by sequencing the human *N-acetyltransferase* genes (*NAT1* and *NAT2*), which code for enzymes that break down various drugs and carcinogens, in 13 groups of people from around the world, he and his colleagues determined that the two genes have led quite different lives down through the ages. As they reported last year, *NAT1*'s DNA sequence has held fairly constant, with few differences seen within and among the groups. But *NAT2*'s sequence varies significantly from group to group, with one variant much more prevalent in people from western and central Eurasia, suggesting that it conferred a survival advantage for the agriculture lifestyles that developed in those regions.

Looking for such selection in *NAT1* and *NAT2* makes sense given that their function is known. But, says Quintana-Murci, "sometimes a gene can be involved in a disease, but you would never have imagined that or never have chosen it in a candidate approach."

So, beginning last year, he broadened his search for evolutionary imprints to the entire genomic landscape. He looked for positive selection across the HapMap, a massive data set chronicling subtle DNA sequence changes among four groups of people around the world, as well as across another survey of human variation among American ethnic groups conducted by Perlegen Sciences Inc. based in Mountain View, California. As expected, the search turned up many genes underlying physical features, such as skin and hair color, as having been selected for, as well as genes involved in fending off pathogens. However, "what's surprising is that we found quite a lot of genes of unknown function that showed extreme differences [between] populations," says Quintana-Murci. "For the moment, we don't know what they are doing."

Quintana-Murci's whole-genome approach is the future of population genetics, he and

most others in the field contended at a recent meeting in France.* But the transition from studying candidate genes to what some call population genomics is proving tricky. Although high-throughput DNA sequencing provides extraordinary amounts of human gene sequence for analysis, population geneticists admit that they are struggling to come up with the statistical tools and theoretical frameworks to make sense of it all. "The data are coming at us so fast that it's hard to keep up," says Noah Rosenberg, a mathematical population geneticist at the University of Michigan, Ann Arbor.

Still, most in the field are confident that they will develop the needed databases and techniques to better handle the explosion of available human DNA sequences. And they anticipate huge rewards as they meet those challenges. "Having a genome's worth of data has completely changed the face of population genetics," says Gil McVean of the University of Oxford, U.K. Laurent Excoffier, a computational biologist at the University of Bern, Switzerland, is even bolder. "These recent developments have meant the end of single [gene] analyses," he contends.

Strength in numbers

For population geneticists, finding where evolution has left its mark in a genome is the pot of gold at the end of the rainbow. They seek genes that have changed at different rates in different populations and use those variations to reconstruct human history. The field has been steeped in theory, with data, especially on humans, dribbling in a few proteins or DNA bases at a time. "The problem with population genetics is there's a lot of hand-waving arguments, but it's difficult to make precise statistical statements" because of a lack of data, says Thomas Bataillon, a population geneticist at the University of Aarhus, Denmark.

Now the genomics revolution, brought about by high-throughput sequencing centers that decipher DNA by the millions of bases a day, promises to put researchers on firmer ground. Having hundreds of gene regions at their fingertips is helping them get a better sense of when evolution underlies the changes in the genome they see. When a particular gene variant confers a survival advantage for a group of people, for example, the resulting positive selection will make it more common in that group than expected by chance alone.

But just because a variant stands out doesn't guarantee that it played a key role in evolution. Twists of fate could have increased its prevalence. Sometimes a small group of people migrate, taking with them only a subset of a gene's variants. As a result, the frequencies of those particular variants in the migrants will likely be inflated compared with those of the source population, but not because of selection. "The main problem is to distinguish what's due to demographics and what's due to selection," says Quintana-Murci.

Here is where strength in numbers comes into play. In theory, any demographic factor—say, a sharp decline in a population—should take a toll on the whole genome, affecting all genes to an equal degree. In contrast, natural selection would affect specific parts, only those few genes in which a particular variant improves a person's chances of survival. Therefore, the more genes population geneticists compare, the better. "With genomewide data, you will get a much better handle on the demography," says Rasmus Nielsen of the University of Copenhagen, Denmark. "We can now much [more] reliably identify regions which have been targeted by selection."

In a few cases, what once seemed an important genetic event evolutionarily speaking has ceased to stand out after comparison to changes across the rest of the genome. Two years ago, researchers discovered skewed distributions for an allele of a gene called *microcephalin* and for a variant of a gene called *ASPM*, both of which regulate brain growth. The research team calculated that a certain *microcephalin* allele appeared around 37,000 years ago, about the same time Europeans began showing symbolic behavior, according to anthropological evidence. The group found this allele in 75% or more of Italians, Russians, and Han Chinese, but in just 30% of the Tanzanian Masaai and in less than 10% of two other African groups tested (*Science*, 9 September 2005, p. 1662). To some, that suggested the allele gave early Europeans a mental boost.

Since then, other researchers looking at more places in the genomes of people from similar populations have called this conclusion into question: The frequencies of that brain-size allele turned out to be not significantly different from frequencies of alleles for other genes seen elsewhere in the genome (*Science*, 20 April, p. 370). That argues against selection for the allele. "The [new] analysis suggests that there is nothing special about this gene, population genetically speaking," says Nielsen.

Rosenberg has also employed genomic analyses to put a particular finding to the test. His team has recently found a novel genetic variant in Native Americans. The variant isn't part of a gene but instead is located at a short repeating stretch of DNA called microsatellite D9S1120, he explained at the meeting in France. (He and Kari Schroeder and David Smith of the University of California, Davis, also reported the data online 13 February in *Biology Letters*.) This variant appears in about one-third of more than a dozen native North and South American groups but very rarely anyplace else in the world except in two groups in Northeastern Siberia.

Rosenberg and colleagues initially thought this variant may have been selected for because it helped the people who came across the Bering Strait and populated North America cope with their new environs. But when the researchers looked more broadly at the population variation in hundreds of other microsatellites, they ruled out natural selection as the reason for the original variant's prominence among Native Americans. The extent of variation for the D9S1120 microsatellite was the same as for other microsatellites not under

Peopling the Americas. A novel gene variant suggests that native North and South Americans (*above*) share a common ancestry.

CREDIT: T.G. SCHURR

* The Jacques-Monod Conference on Evolutionary Genomics, 2–6 May, Roscoff, France.

selection. "Colonization of the Americas from a small founding population provides a much better explanation of the pattern," says Rosenberg.

Still, even without being a target of selection, the microsatellite originally found by Rosenberg and colleagues has proven interesting. Some researchers propose that multiple waves of migrants from different parts of Asia moved into the Americas, citing the existence of different language groups as evidence. The microsatellite's similarly high frequency in all Native Americans, however, suggests a single ancestry, adds Rosenberg.

Surfing on a genome

Although population genomic approaches are illuminating new leads and eliminating false ones, Rosenberg and others are finding they sometimes need to temper their enthusiasm. Until sequencing of entire individual genomes becomes feasible at a reasonable cost and effort, whole-genome data sets are not quite "whole," and that incompleteness can trip up researchers. For the current HapMap, the problem lies in what went into the survey. Because the single-nucleotide polymorphisms (SNPs) cataloged in HapMap initially came from studies of just a few dozen people, they include only the most common of these base differences. But population geneticists need to be able to evaluate the full repertoire of SNPs, including rare base changes, in order to interpret the patterns of variants they see. They can adjust their analyses to factor in a bias toward common SNPs, if they know exactly how those SNPs were found. But HapMap's researchers used a variety of criteria, sometimes relying on human-chimp differences, other times on human-human differences, to identify the SNPs, making it virtually impossible to compensate for the bias.

"There are a number of studies that cannot be done because of the problems with the data," Nielsen complains, noting that the original 2005 HapMap paper failed to address this bias adequately in its initial results. Nielsen predicts population geneticists will be better off once sequencing costs drop enough for researchers to sequence entire genomes of the people they study: "Although this data will have its own problems, it will allow the field of human population genetics to finally move forward and put the problems of ascertainment biases behind us."

Researchers are also discovering that the idea that demographic history affects the entire genome equally is too simplistic, thus limiting the ability of genomic analyses to positively identify that a gene variation's prominence is the result of natural selection. Excoffier has been studying a phenomenon called "surfing," which he thinks could also explain the apparent increase in frequency of brain-size genes in certain populations (*Science*, 14 July 2006, p. 172). On the ocean, surfers catch a ride on the front of a wave; "surfing" in a genomics sense refers to gene variants harbored by the leading edge of a population that is expanding into new territory. Computer simulations show that individuals on this leading edge will sometimes simply by chance be more prolific than individuals closer to the home base. This leads to a disproportionate increase in any variant possessed by the trailblazers—even though the variant might not enhance survival in any way. Thus, even when the change in frequency affects just one gene, "we can't be sure it's selection any more," says Excoffier.

Finally, the genome itself can play tricks on population geneticists, as processes such as recombination also shape allele frequencies. McVean has been tracking down the DNA sequences where chromosomes pairs recombine, swapping equivalent pieces of DNA. With the compiling of the HapMap, researchers realized that such recombination occurs in specific hot spots. Almost half of the 30,000 recombination sites now identified are packed into 3% of the genome. These hot spots can play havoc with attempts to determine how long ago any nearby gene variant arose, a key step in determining whether that variant is undergoing selection. "It's important to take [hot spots] into account," says Laurent Duret, a computational biologist at Claude Bernard University in Lyon, France.

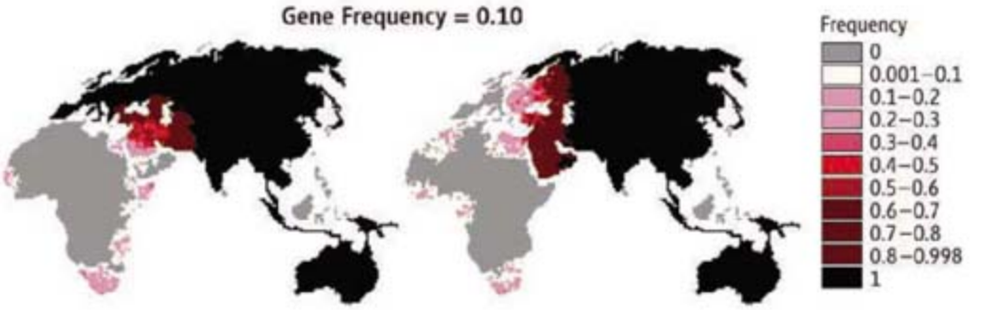
Recognizing that need, McVean and his colleagues in 2005 figured out that hot spots often included a signature DNA sequence. At the meeting, McVean described a second signature sequence, located just a few bases from the first one he and his colleagues found. He found that second sequence by analyzing new SNP data collected as part of the second phase of the HapMap project. The two signatures exist in 40% of the hot spots, and there, recombination is 20 times more likely to occur than at an average spot on a chromosome. These results should help them and others better factor in the effect of recombination as they evaluate the age and importance of particular alleles.

As population genetics becomes population genomics, researchers need to come up with better theories for analyzing the patterns they detect. "To interpret all the variation we see, we have to develop models of sequence evolution, taking into account demography and molecular processes" such as recombination, says Duret. In addition, population geneticists are desperate for better statistical methods for studying lots of genes at once. "If we are to make use of the kind of data provided by this new technology, we will need a rigorous statistical basis for its analysis," says Darren Obbard of the University of Edinburgh, U.K. Researchers such as Nielsen are working on new methods, but they still have a long way to go to make these approaches easy for the rest of the field to use. "We're lacking simple, efficient software," says Thierry Wirth of the Natural History Museum in Paris.

The genomic gold rush may pose other problems, too. As the sequencing of individual genomes becomes more common, the demands on data storage will skyrocket. And, says Excoffier, "to be really sure that selection is acting, one would need to get some functional evidence" for how a gene variant aided people. But, he notes, genes identified through these large-scale studies are often not well characterized.

Nonetheless, the promise of cheap sequencing and of data sets that cover most if not all of the human genome is great for population geneticists. With them, says Rosenberg, "we'll be able to get closer to the limits of what's possible to know about human evolutionary history on the basis of genetics."

–ELIZABETH PENNISI



Chance occurrence. A computer simulation shows that over the 1400 generations it took for Africans to expand into and populate Europe and Asia, gene variants can by chance increase quite a bit in frequency but to varying degrees in different places.

CREDIT: CURRAT *ET AL.*, *SCIENCE* (2006)

CREDIT: CORBIS

Invisible threat. U.S. intelligence agencies say spotting missile sites in Cuba in 1962 was technologically easier than today's task of snooping on terrorists.

U.S. SCIENCE POLICY

Congress Splits Over Plan to Consolidate Intelligence Research

U.S. intelligence agencies need new surveillance tools to fight global terrorism. But it's not clear how they should carry out the necessary research

In October 1962, U.S. reconnaissance airplanes provided evidence that the Soviet Union was building up an arsenal of warheads on Cuba, only 150 km off the Florida coast. Those pictures led to high-level talks between the two superpowers that averted what many believe could have been a nuclear war.

In hindsight, intelligence experts say that finding missile sites was a piece of cake compared to the surveillance challenges in the post-9/11 world. Unlike during the Cold War era, they say, intelligence agencies today must track not only government military installations but also terrorist networks and individuals. To meet that challenge, the Director of National Intelligence (DNI), Michael McConnell, has proposed cobbling together existing U.S. research and development (R&D) programs at 14 agencies into a new organization. Modeled on the Defense Advanced Research Projects Agency (DARPA), the proposed Intelligence Advanced Research Projects Activity (IARPA) would be built mainly from merging the Intelligence Technology Innovation Center at the Central Intelligence Agency (CIA), the Advanced Research and Development Activity (ARDA) at the National Security Agency (NSA), and the National Technology Alliance at the National Geospatial-Intelligence Agency.

McConnell says the new arrangement will stimulate long-range research on the gathering and analysis of intelligence that now falls outside the mission of a particular agency. "We are in a rut right now, turning the crank on the same technologies," says IARPA's acting director Steven Nixon. "What we need to do is swing for the fences."

But Congress is divided over the plan. The House version of the 2008 Intelligence Authorization Act passed last month contains language that forbids DNI from merging any existing research programs under IARPA. "They are creating just another agency looking for a piece of the pie that will push their own pet rocks," says Representative Heather Wilson (R–NM), who thinks that McConnell's office should instead continue to coordinate programs run by each intelligence agency. Wilson and others worry that the new organization might end up subsuming the entire science and technology operations of individual agencies, leaving them with no science portfolio of their own.

Two weeks later, however, the Senate endorsed the idea when it passed its version of the overall bill. "We think IARPA can fill in gaps between the needs of single agencies," says a Senate aide, who expects the plan to survive when the two bills go to conference this fall. "It's an invalid concern that IARPA is suddenly going to become the program manager for all the science that's done by the intelligence community."

DNI officials say the new agency, with its 40 program managers headquartered in leased space at the University of Maryland in College Park, is needed to keep pace with the rapidly evolving threat of global terrorism. IARPA would sponsor basic and applied academic research on intelligence-related topics such as machine translation of foreign languages, pattern recognition, and quantum encryption with grants to academia, national labs, and industry.

Although President George W. Bush's fiscal year 2008 budget request asks Congress for only a modest increase in 2008 over the science budgets of IARPA's constituent programs, DNI officials hope for a 5-year doubling of current budgets. The size of the budget is classified, but outside experts speculate that the intelligence agencies are now spending between $250 million and $350 million on the programs IARPA would consolidate.

Researchers seem enthused by the plan. "It seems likely to me that an integrated approach to tackling 'grand challenge'–type R&D could yield a greater return on investment," says Ruth David, an electrical engineer who once led CIA's science and technology directorate and now runs Analytic Services in Arlington, Virginia.

Current programs funded by CIA or NSA strive to develop products that can "immediately plug into existing intelligence analysis systems," says Mark Steyvers, a computer scientist at the University of California, Irvine, who has done unclassified research for the CIA on extracting meaningful text from huge data sets such as e-mail chatter on the Internet. But he says those products lack broader applicability. Steyvers predicts that IARPA would spark "exciting new collaborations" between disciplines such as computer science, statistics, and the social sciences.

IARPA plans to seek exactly those kinds of interdisciplinary proposals, says Nixon, with an eye toward decoding chat-room conversations between terrorists and monitoring weapons and people in otherwise inaccessible regions. "During the Cold War," says Nixon, "we had big monolithic targets, and so we needed technologies to answer questions like, 'Where's the airfield, and how many bombers do they have?' Now, the targets have shrunk, and they are all over the place."

–YUDHIJIT BHATTACHARJEE

LETTERS

edited by Etta Kavanagh

The Utility of Standardized Tests

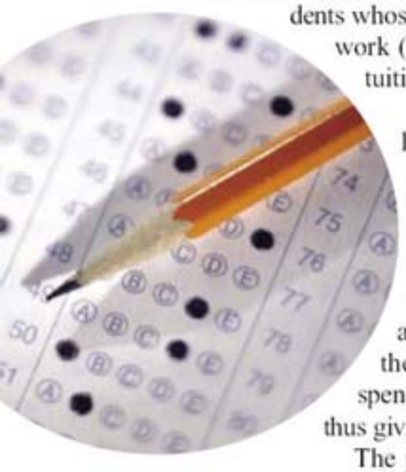
IN THEIR EDUCATION FORUM "STANDARDIZED TESTS PREDICT GRADUATE STUDENTS' success" (23 Feb., p. 1080), N. R. Kuncel and S. A. Hezlett synthesize the results of studies examining standardized test scores and performances in graduate school. They conclude that standardized tests such as the GREs are good predictors of professional success. They consider and reject possible biases such as racial, ethnic, and/or gender groups. They also take into account the possibility that the evaluators of success (for the subjective measures) might be biased by their knowledge of individuals' test scores. Kuncel and Hezlett do not, however, consider the causal basis for their observed correlations.

An underlying assumption to their study is that, given the control for the above-mentioned biases, any observed correlations between test scores and success are due to causal links between aptitude and test score and between aptitude and performance. That is, they postulate two parallel causal chains:

Greater aptitude→Higher scores **AND** *Greater aptitude→Better performance*

Kuncel and Hezlett do not, apparently, account for other possible causal links between test score and professional success that could affect the interpretation of their observed correlations. Such causal connections could create strong correlations in which the role of test score is quite real but occurs because of the way external actors (not the students) react to such scores. This situation involves an indirect, though distinctly causal, role for test scores.

One such indirect causal link is the fact that students with high GRE scores tend to be the ones who receive better support in the forms of fellowships, plum research and/or teaching assistantships, and, in some cases, direct support from the university for their research as part of their recruitment package. This superior financial support allows them to get a head start on their careers, publish more, get better jobs, and, in many ways, outperform those students whose support levels are lower and/or who have to devote time to paid work (e.g., teaching) during graduate school to provide funding for tuition and living expenses.



A related point is that there are studies that show that a "gifted" label for students can be self-fulfilling. There have been numerous discussions in the education literature as to the extent to which teachers' expectations can influence students' performances on intelligence or achievement tests and similar studies in the workplace literature on supervisors' effects (*1*–*3*). This instructor or supervisor effect may be an important point for graduate performance as well; faculty members will probably clamor to work with the students who seemed most attractive in admissions and may spend more time with those students than with the lower-scoring ones, thus giving the high scorers a leg up beyond financial advantages.

The phrase "correlation is not necessarily causation" has become ingrained among those scientists whose work involves correlative analyses. An equally important, although less loudly trumpeted, truism is that different causal links can lead to similar correlative relationships. In the case of test scores and professional success, the causal paths may be the ones apparently implied by Kuncel and Hezlett. Alternatively, the true chain of causality may be

Higher scores→Better intellectual and/or financial support→Better performance

Obviously, these different causal mechanisms suggest very different policy responses to the observed correlations between scores and performances. A challenge is to try to understand how fellowship and graduate programs treat applicants and students with different test scores and how that treatment might lead to different performance outcomes. Kuncel and Hezlett take an important first step toward this understanding in demonstrating the strengths of observed correlations, but their study says nothing about the actual causal chains at work.

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References

1. E. E. Jones, *Science* **234**, 41 (1986).
2. R. Rosenthal, *Educ. Res.* **16**, 37 (1987).
3. N. Kierein, M. Gold, *J. Organ. Behav.* **21**, 913 (2000).

N. R. KUNCEL AND S. A. HEZLETT PRESENT AN enthusiastic case for standardized graduate school admissions tests. Their terms "predict" and "success" are problematic. They show a 0.4 correlation between the test scores and first-year graduate grades and between test scores and other graduate grades, but 0.4 is a limited predictor of an effect. The study shows much lower correlations between test scores and research productivity, citation counts, and degree completion. The essence of a successful graduate program is the production of an able professional, not someone who passed a few courses.

The authors concluded with more moderate statements that standardized tests are useful predictors of subsequent performance in graduate school and that they have positive and useful relationships with accomplishments. Their findings are more appropriate to master's programs, for which grades are the major requirements.

For doctoral programs, class grades are a smaller part of the picture. Test data on what a college student remembers cannot predict how the student will use new information to think in creative and original patterns, manage complex research tasks, and explore the unknown. Completing a doctoral dissertation


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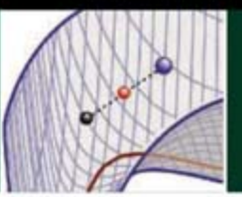
Reaction dynamics
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requires financial resources, social skills, aggressiveness, creativity, persistence, resilience, managerial skills, motivation, ability to work independently, family stability, health, and luck. There is a need for qualitative measures that predict student–graduate program compatibility.

The real purpose of admissions tests is to judge student applications at the cheapest possible price that justifies acceptance or rejection. An interesting aspect of the data presented is that the Miller Analogies Test (MAT) predicts graduate grades as well as the six more expensive tests such as the Graduate Record Examination (GRE). If we must test, why not go the cheap and simple route?

BERNARD BROWN

Rockville, MD, USA.

THE EDUCATION FORUM BY N. R. KUNCEL and S. A. Hezlett is scientifically unsound and socially reprehensible. The authors, editors, and approving reviewers must all bear responsibility for publication of a report that is fundamentally flawed, but that, if unchallenged, could set back hard-won progress toward reducing unfair discrimination in graduate school admissions by decades. Members of admissions committees who are prone to unfairly discriminate against underrepresented graduate school applicants may use Kuncel and Hezlett's work to justify excluding students solely on the basis of the authors' erroneous assertion that test scores commonly evaluated for graduate school admission predict future graduate and postgraduate performance. Kuncel and Hezlett write, "Accurately predicting which students are best suited for postbaccalaureate graduate school programs benefits the programs, the students, and society at large, because it allows education to be concentrated on those most likely to profit." Even if standardized testing could identify students who were "most likely to profit" from a graduate education, only a crude, backward society would actively seek to limit opportunity in this manner. However, standardized testing cannot do this. Kuncel and Hezlett make the elementary error of equating aggregate correlations with predictive power. Nothing in their analysis permits an admissions committee to look at an applicant's test scores and validly predict what that student would accomplish in the graduate program or their career thereafter. In fact, even their misrepresented correlation analysis has obvious flaws. No attention is given to the likelihood that the specific test score distributions of unfairly discriminated groups will differ substantially from that of the larger majority group whose distributions predominate in the correlation data. Finally, the misrepresented correlations are themselves over-

stated by the authors. More than half of the variance in "later performances" cannot be attributed to the observed variance in standardized test scores.

JAMES L. SHERLEY

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Response

LERDAU AND AVERY RAISE THE IMPORTANT issue of the nature of the causal links between test scores and student performance. Clearly, scientists cannot directly manipulate GRE scores to conduct formal experiments. We must rely on a convergence of evidence to draw inferences about causality. While acknowledging that higher test scores may foster more support, there are many pieces of evidence that support the conclusion that test scores measure ability and skills and that these abilities and skills lead to learning and performance.

First, the abilities assessed by standardized tests have been repeatedly shown to be predictive of complex skill acquisition in field and laboratory studies where test scores are not known to either the subject or the experimenters (*1*). Second, these abilities are associated with specific brain structures and have been shown to have both a sizable heritable and environmental component (*2*, *3*). Both sets of findings have been replicated across multiple studies using different research designs and methods.

Third, there is a literature on graduate students that directly speaks to the questions raised by Lerdau and Avery. U.S. National Science Foundation (NSF) Fellows are evaluated and classified into categories based on ratings of capability. All students in the top category are awarded fellowships. Approximately half of the students in the second category are awarded fellowships based on administrative concerns (e.g., the distribution of awards across fields).

Within the second category, the treatment of receiving a fellowship yields an effect for degree attainment (*4*). It is indeed true that after holding ability effectively constant, the fellowship has an effect, quite possibly due to both resources and expectations. However, the magnitude of this effect was 0.07 for degree attainment in contrast to 0.22 for the GRE and 0.39 for the GRE Subject tests. A second study compared students who all received a NSF Fellowship but were either in the first or the second ability categories (*5*). A student's actual category placement is not reported to students or universities. Therefore, this study controls for a very large and prestigious environmental resource that would (if anything can) produce sizable expectation effects. Even with the same fellowship in hand and despite tremendous restriction of range in ability, those in the first category were more likely to finish the Ph.D., attain faculty status in a high-ranked department, and win grants than those in the second category. Effects for both studies are of practical importance but modest size, with generally a 2 to 7% increase in positive outcomes.

Finally, Lerdau and Avery suggest that the literature they cite applies to graduate school programs. We are inclined to agree to some extent, but note that their citations are based on a quasi-experimental contrast between performance outcomes for one group (typically children or military personnel) that is falsely presented to a teacher or supervisor as having demonstrated superior aptitude versus a second generally equivalent control group. This is not the same as the hand-picked cohorts of students in doctoral graduate programs where the contrast would be between the upper and lower halves of a rather homogeneous group. Would telling faculty that one group of students scored an average of 1500 versus another group with an average of 1400 really produce the same and often modest (0.15 for academic performance) expectation effects? We conclude that high-ability students brought into an environment with high expectations and good resources are likely to be the best recipe for success. Success is not simply a function of resources.

Test score	Earn Ph.D.	Drop out
Above average	60%	40%
Below average	40%	60%

We are in complete agreement with Brown that the "essence of a successful graduate program is the production of an able professional." We also agree, and noted in our paper, that there are other important applicant characteristics that are, unfortunately, poorly measured by letters of recommendation and the like, including the drive and motivation that Brown mentions. However, we strongly disagree that the tests do not predict much more than grades.

Brown overlooks some key findings. The one that speaks most directly to his Letter are the results for faculty ratings. These are faculty evaluations of a student's performance as a researcher, the quality of their dissertation research, their professionalism, and so on. The correlations here range from 0.40 to 0.50. In a separate study, we also demonstrated that standardized tests are predictive of faculty and supervisor evaluations of creativity, career potential, and job performance (*6*). We are clearly improving our chances of producing more able professionals when tests are associated with an increase in the level of these evaluations as well as research accomplishments; good performance on comprehensive, qualifying, and licensing examinations; an increase in the odds of finishing; and, yes, better grades.

Brown's Letter also suggests that these relationships are not very large. First, when it comes to predicting complex human behavior, there are few effects that are stronger (*7*). Second, our experience is that many researchers become accustomed to the size of effects in their field and don't really see the utility of even small correlations. A brief illustration can help clarify the utility of a 0.40 correlation. For simplicity's sake, imagine that we have dichotomized test scores at the median into above- and below-average groups with an outcome of earning versus failing to earn the Ph.D. Sixty percent earn the Ph.D in the above-average group while 40% do so in the below-average group. How large is the correlation (see table)?

This is a correlation of just 0.20. Most of the results we present are much larger, yet even this correlation yields a 20% difference in degree completion. Small gains, across multiple outcomes, year after year, improve the strength and health of a program.

Setting aside the hyperbole, Sherley's substantive concerns appear to focus on the legitimacy of controlling access to higher education programs, the implications of our findings for discriminatory admissions decisions, the appropriateness of using meta-analysis of correlations, and the influence of score distribution differences in our analyses.

We would love to live in a world with unlimited resources where all can go to the graduate program of their choice and pursue the career of their choice. Unfortunately, opportunities and resources are limited, and decisions must be made about whom to admit given limited faculty, funding, and space. Neither crude nor backward, this has always been and will always be reality.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

If anyone is using our research to justify discriminatory practices, they should stop, because it does not provide any justification for such practices. Exceptional talent exists in all groups and should be sought and nurtured. The results suggest that score differences are symptomatic of other social and societal factors and are not the result of the tests themselves.

Our research is based on standard inferential statistics used in the social, physical, medical, and biological sciences. The correlation is transformable into many other effect sizes and test statistics and quantifies in our study the relationship between a variable measured at one time and an outcome measured at a later time. This is a predictive relationship. The correlations are aggregated using meta-analytic methods that have been subjugated to numerous simulation studies and are used, again, in numerous fields to quantify predictive effects (*8*–*10*).

Our analytic methods are solid, and Sherley provides no evidence or citations to indicate otherwise. That Sherley can in one sentence argue that our methods do not yield an estimate of predictive power and in another argue that the estimate of predictive power is too weak suggests that something is amiss with his argument and logic. This criticism is so puzzling, we wonder if Sherley believes that we present weighted correlations between means, which would be inappropriate, instead of the weighted mean of correlations that comprise our data.

Sherley is also utterly wrong that we give no attention "to the likelihood that the specific test score distributions of unfairly discriminated groups will differ substantially from that of the larger majority group." We explicitly discuss research (over 20 citations) that examines relationships for racial and gender groups separately both in the article and the SOM. This research finds that the relationship between test scores and academic performance is effectively the same across groups. This fact is recognized by some of the strongest advocates of affirmative action (*11*).

The items in standardized tests measure making decisions after interpreting a table of data, using algebra to solve a problem, reading a passage and drawing inferences from the text, and knowledge of core discipline–specific concepts. These are valuable. Sherley's Letter effectively argues that objective assessments of these skills should not be a part of graduate admissions because members of some groups currently do not do them as well as other groups. We feel that this is the most damaging and unsound position of all, because it is so very clear to us that they can.

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References

1. P. L. Ackerman, *Psychol. Bull.* **97**, 129 (1985).
2. J. R. Gray, P. M. Thompson, *Nat. Rev. Neurosci.* **5**, 471 (2004).
3. T. J. Bouchard *et al.*, *Science* **252**, 191 (1991).
4. G. B. Chapman, C. McCauley, *Educ. Psychol. Meas.* **54**, 428 (1994).
5. G. B. Chapman, C. McCauley, *J. Appl. Psychol.* **78**, 815 (1993).
6. N. R. Kuncel, S. A. Hezlett, D. S. Ones, *J. Pers. Soc. Psychol.* **86**, 148 (2004).
7. D. C. Funder, C. R. Colvin, *J. Pers. Soc. Psychol.* **60**, 773 (1991).
8. L. V. Hedges, I. Olkin, *Statistical Methods for Meta-Analysis* (Academic Press, New York, 1985).
9. J. E Hunter, F. L. Schmidt, *Methods of Meta-Analysis* (Sage, Thousand Oaks, CA, 2004).
10. R. Schulze, *Meta-Analysis: A Comparison of Approaches* (Hogrefe and Huber, Göttingen, Germany, 2004).
11. W. G. Bowen, D. Bok, *The Shape of the River* (Princeton Univ. Press, Princeton, NJ, 1998).

CORRECTIONS AND CLARIFICATIONS

News of the Week: "Meanwhile, back in Washington…" by E. Kintisch (11 May, p. 813). On the diagram, the emissions path of 167 billion metric tons should be labeled "Waxman Plan." The McCain-Lieberman plan lies between the 203-billion-metric-ton and 287-billion-metric-ton curves.

Policy Forum: "Environmental biology and human disease" by D. Schwartz and F. Collins (4 May, p. 695). The authors' affiliations and contact information were omitted. The authors are with the National Institutes of Health, Bethesda, MD 20892, USA. David Schwartz is the author for correspondence. E-mail: david.schwartz@niehs.nih.gov

Research Articles: "Signals from chloroplasts converge to regulate nuclear gene expression" by S. Koussevitzky *et al.* (4 May, p. 715). The title was shortened for print, but it should have remained the same as it was originally published online: "Multiple signals from damaged chloroplasts converge on a common pathway to regulate nuclear gene expression."

News Focus: "Killing whales for science?" by V. Morell (27 Apr., p. 532). On page 534, the story paraphrased Lars Walløe as saying that Japanese genetic data were important in estimating minke whale numbers. Walløe actually cited genetic and other types of Japanese data as valid, and described the nongenetic data as being important in estimating minke numbers.

Reviews: "The problem with determining atomic structure at the nanoscale" by S. J. L. Billinge and I. Levin (27 Apr., p. 561). The reference to the source article for the TEM image of nanoparticles in Fig. 1C was omitted. The image was adapted from a figure in J. R. McBride, T. C. Kippeny, S. J. Pennycook, S. J. Rosenthal, *Nanoletters* **4**, 1279 (2004).

Reports: "Protein sequences from mastodon and *Tyrannosaurus rex* revealed by mass spectrometry" by J. M. Asara *et al.* (13 Apr., p. 280). Two authors' affiliations were listed incorrectly. John M. Asara is at the Division of Signal Transduction, Beth Israel Deaconess Medical Center, Boston, MA 02115, USA; and the Department of Pathology, Harvard Medical School, Boston, MA 02115, USA. Mary H. Schweitzer is at the Department of Marine, Earth and Atmospheric Sciences, North Carolina State University, Raleigh, NC 27695, USA; the North Carolina Museum of Natural Sciences, Raleigh, NC 27601, USA; and Museum of the Rockies, Montana State University, Bozeman, MT 59717, USA.

Special Section on Freshwater Resources: Perspectives: "Seeking sustainability: Israel's evolving water management strategy" by A. Tal (25 Aug. 2006, p. 1081). Figure 1, the schematic drawing of the National Water Carrier course, was inaccurate. A more precise map is available online at http://www.sciencemag.org/cgi/reprint/313/5790/1081.pdf (see last page).

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Tequila, a Neurotrypsin Ortholog, Regulates Long-Term Memory Formation in *Drosophila*"

Peter Sonderegger and Laszlo Patthy

Didelot *et al.* (Reports, 11 August 2006, p. 851) claimed that *Drosophila* Tequila (Teq) and human neurotrypsin are orthologs and concluded that deficient long-term memory after Teq inactivation indicates that neurotrypsin plays its essential role for human cognitive functions through a similar mechanism. Our analyses suggest that Teq and neurotrypsin are not orthologous, leading us to question their equivalent roles in higher brain function.

Full text at www.sciencemag.org/cgi/content/full/316/5832/1698b

RESPONSE TO COMMENT ON "Tequila, a Neurotrypsin Ortholog, Regulates Long-Term Memory Formation in *Drosophila*"

Thomas Preat, Jean-Luc Da Lage, Laurence Colleaux, Gérard Didelot, Florence Molinari, Paul Tchénio, Elodie Milhiet, Arnold Munnich, Marie-Louise Cariou

Sonderegger and Patthy argue that the trypsin catalytic domains of *Drosophila* Tequila and human neurotrypsin are not linked by an orthology relationship. We present analyses based both on BLAST (basic local alignment search tool) comparisons and on phylogenetic relationships, which show that these two proteases do share an orthologous region that includes the trypsin domain.

Full text at www.sciencemag.org/cgi/content/full/316/5832/1698c

ECOLOGY

Remodeled Foundations

Simon A. Levin

When I first met Robert May 35 years ago, theoretical ecology as a subject did not exist. Admittedly, today's textbooks build heavily on much older theoretical work, especially that of the intellectual giants Vito Volterra and Alfred Lotka. Furthermore, the quantitative core of the subject is drawn from early developments in demography, population genetics, and epidemiology as well as in the description of the distribution and abundance of species. But Volterra and Lotka were not primarily ecologists; their contributions to the subject, fundamental as they have been, were sidelines for them. So, too, the pioneers in demography (which sensu strictu refers to the dynamics only of human populations), population genetics, and epidemiology were little concerned with ecology. And Frank Preston, whose famous papers on ecological abundance remain central to the field, was first and foremost an engineer who made his living as a consultant to the glass industry.

Theoretical Ecology
Principles and Applications. 3rd ed.

Robert M. May and Angela R. McLean, Eds.

Oxford University Press, Oxford, 2007. 267 pp. $120, £60. ISBN 9780199209989. Paper, $45, £29.95. ISBN 9780199209996.

The field has come a long way in the years since, and no one played a larger role in that growth than May. *Theoretical Ecology*, a well-edited compilation of contributions by some of the many people he has influenced directly in his career (including his co-editor, Angela McLean), is testimony to both the maturation of the subject and May's own role in that process. Thirty-five years ago, ecological problems were little more than amusements for mathematicians and physicists, who were attracted by the misleading simplicity of models and too often had little real interest in the biology. Today, the subject of ecology is rich in quantitative reasoning, built on a strong theoretical foundation, in large part because of an infusion of mathematicians and physicists who—enchanted not with the simplicity but with the elegant complexity of the subject, and deeply committed to the fundamental ecological and evolutionary issues—became biologists. In the vanguard of this transformation was Bob May, whose diverse contributions not only helped build a foundation for theoretical ecology but also forced demography, epidemiology, and genetics to recognize that they too must cast their problems within an ecological framework.

Do not be misled by the fact that the book is billed as the third edition. It bears very little resemblance to its predecessors, and indeed the trilogy is a must for every ecologist's library. The subject has evolved a great deal in the past three decades, and May and McLean recognized that in their choice of subjects and authors: All but two contributors (beyond May himself) are new since the previous edition. The topics range from the basics (models of single-species and interacting populations) to applications (fisheries, conservation biology, infectious diseases, and food production). There is no attempt to touch all bases, and rich parts of theoretical ecology such as autecology, animal movement, and ecosystems are barely mentioned. Nonetheless, the coverage provides a broad introduction to current literature of theoretical ecology and a superb integration of theory and fact.

The authors of the diverse chapters have done outstanding jobs of introducing the core material while providing entertaining and highly readable discourse; somehow, despite the diversity of authors, the whole thing hangs together. I am delighted that the book has appeared when it has, because it will be an essential text when I next teach my course in theoretical ecology; I expect that it will become a standard text for many such courses. Nonetheless, the appeal should be much broader: The book will also serve as an engaging introduction for anyone interested in learning about the theoretical foundations of much of ecology.

The volume's broad scope reflects the equally broad range of topics that May has influenced, and he could well have written any of the chapters himself. His contributions to the development of the theory of chaotic dynamics are well known and discussed in the chapter by Tim Coulson and Charles Godfray on single-species dynamics. His seminal work on the relationship between diversity and stability is similarly chronicled in the chapter by Tony Ives. And his foundational collaborations with Roy Anderson, firmly embedding the theory of infectious diseases within an ecological framework, are expertly reported in the chapter by Bryan Grenfell and Matt Keeling. I was thus intrigued by the chapters May left for himself, because I suspect that these are the ones closest to his heart. Beyond the introduction with Angela McLean, there are two. "Communities: patterns," written with Mick Crawley and George Sugihara, is perhaps the most integrative chapter in the book. It unifies classical and modern work on species abundance distributions, food webs, species-area relations, and community dynamics, relating these to the flow of energy and materials. For me, this chapter represents what May has long been working on: building a synthetic theory that shows the unity of the diverse windows on ecological interactions that come from investigations at different levels of organization.

I suspect, however, that the last chapter

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Andy Goldsworthy's *Dandelion Line* (2000).

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("Unanswered questions and why they matter") is the one that May most wanted to write, because it shows how far the field has come. Initially a distinguished theoretical physicist and then a leading theoretician in ecology, May became the chief scientific adviser to the Crown and later the president of the Royal Society. In parallel, and not just coincidentally, theoretical ecology grew from abstraction to application, becoming an essential tool in addressing the sorts of problems that fill the chapters of this book. But now we face challenges greater than ever before because of their global scope: climate change, the loss of biodiversity, the emergence of novel infectious diseases, and the sustainability of the services ecosystems provide humans. Dealing with these rests on developing a more powerful theoretical ecology, reaching beyond the foundations that have been built to challenges such as understanding how cooperation can be achieved in a global commons and attaining a broader integration of ecology with the social sciences and the humanities. These issues have to a large extent occupied May's efforts for the past decade and are a fitting finale to the book. The last chapter reminds theoretical ecologists that their work has just begun.

10.1126/science.1141870

PLANT SCIENCE

Explaining Floral Diversity

Beryl B. Simpson

One of the main functions of flowers is to attract animals. It is therefore no wonder that humans are fascinated by flowers. Theophrastus discussed floral characters, Linnaeus based his classification of plants on floral form, and Darwin devoted several books and articles to the forms and functions of flowers. Even Goethe, in addition to his poetic and musical offerings, felt compelled to explain the origins of floral structures. It has traditionally been assumed that selection by pollination vectors, principally animals, has produced the myriad of shapes, colors, arrangements, and rewards that we now find across the angiosperm spectrum. Much of the study of floral biology through the first half of the 20th century focused on flower form and the pollinating agents considered to be the driving forces behind floral morphological diversity.

One might therefore expect to find in a book titled *Ecology and Evolution of Flowers* an extensive coverage of pollinators. However, they are explicitly the subjects of only one chapter. One might also expect to find a discussion of recent phylogenetic insights into the changes in floral form from primitive to advanced taxa, a chapter highlighting the paleobotanical evidence of the origins of flowers, or a chapter on the genetics that govern the development of floral form. None of these appears in this book. What, then, is *Ecology and Evolution of Flowers* about? It is a book about angiosperm reproductive biology. Flowers house the reproductive systems of these plants, and thus the word in the title can be construed as an inclusive metaphor for the many aspects of flowering plant reproduction.

Ecology and Evolution of Flowers

Lawrence D. Harder and Spencer C. H. Barrett, Eds.

Oxford University Press, Oxford, 2006. 398 pp. $150, £75. ISBN 9780198570851. Paper, $75, £37.50. ISBN 9780198570868.

In the later half of the 20th century, biologists began to explore aspects of floral biology beyond pollination syndromes. This new approach to plant reproductive biology was pioneered by the late David Lloyd, professor of plant sciences at the University of Canterbury, New Zealand. *Ecology and Evolution of Flowers* is unabashedly a celebration and extension of Lloyd's work. The first chapter offers a mini-biography of his life along with a synopsis of the areas to which he made major contributions: self- and cross-fertilization in plants, gender strategies, allocation strategies, and floral mechanisms. Chapters by an international array of plant biologists are placed in sections that reflect these areas.

Many of the chapters are theoretical, with equations, tables, and graphs far outnumbering pictures. In fact, except for the photographs on the cover and in six of the eight clustered colored plates, the entire book contains no drawings and only one other picture of a flower. The authors assume that we are familiar with the dazzling array of floral forms and that what we really want to know about are the selective influences responsible for this diversity. Beyond pollinators, abiotic factors (such as microhabitat conditions, water or nutrient stress, human disturbance, elevation, and the timing and length of snow melt) and other biological factors (such as herbivores, pathogens, and the composition of the co-flowering plant community) are implicated as selective agents acting on floral phenology, morphology, and breeding systems. These various agents are discussed from theoretical points of view and with examples from the personal research of the authors.

Lloyd was among the first to emphasize the need to consider gender function as a major factor driving the form and behavior of individual flowers. Several chapters look at the contrasting or complementary needs for allocation to male or female function within or between flowers. These needs underlie herkogamy (the spatial segregation of dehiscing anthers and receptive stigmas within flowers) and the myriad of gender polymorphisms—some of which lead to the ultimate in gender specialization, dioecy (populations composed of separate female and male plants). Both Lloyd and Spencer Barrett, one of the editors, have devoted much of their research to such floral asymmetries, so it is not surprising that three chapters focus on this aspect of floral biology. Other contributors use the concept of sex allocation in cosexual flowers as a means to predict minimum size for initial reproduction, potential correlations between male and female allocation, why protandry (male before female) is more common than protogyny (vice versa), the shifts from open pollination to specialist pollination systems, and those between selfing and outcrossing systems.

Diversity in floral form has also been linked to speciation. Several chapters explore this relationship by examining the within-species floral variation across geography and the role of hybridization in selection for divergence in floral form. The roles of key innovations—such as modes of pollination, the advent of nectar spurs, floral asymmetry, and dioecy—in explaining increased (or decreased) speciation are examined from a phylogenetic perspective using contrasts between clades that possess and clades that lack the innovations.

The volume's 18 chapters provide the most in-depth compilation to date on the forces thought to shape the evolution of the structure of angiosperm reproductive organs and mating systems. They include information about almost any aspect of the floral ecology and reproductive biology of flowering plants. Most of the authors also suggest future explorations or empirical demonstrations that will improve our understanding of how and why we have such a diversity of floral morphologies and reproductive strategies.

10.1126/science.1144393

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SCIENCE AND GOVERNMENT

Taking Science Out of the Box—Foresight Recast

The latest form of the U.K. Foresight program demonstrates that the ability to make decisions based on scientific evidence is something no government should be without.

David A. King and Sandy M. Thomas*

Science belongs at the heart of good government, but too often it is relegated to the political sidelines. The problem comes from both sides: scientists who do not know how to convey their expertise to a wider world and politicians who are not convinced that it is worth their while to listen. For the past 5 years, a new program has been running in Britain that aims to bridge this gap. The program has demonstrated that when scientists speak clearly to a receptive political audience, the result can be highly effective.

The recast Foresight program was born out of a time of crisis. In early 2001, foot-and-mouth disease had just begun to grip Britain. The country was filled with burning pyres and fearful farmers. One of us (D.A.K.) had just been appointed Chief Scientific Adviser to the British Government and rapidly assembled a team of epidemiological modelers, virologists, and logistics modelers to determine why the disease was spreading so rapidly and to make recommendations about how to contain it. The team's models suggested that culling of ill animals from a herd should begin within 24 hours after a veterinarian could confirm a farmer's suspicion that the disease was present (*1*, *2*). Animals in any neighboring farms should also be culled as soon as the disease in the first farm had been confirmed by laboratory analysis. Within a few days, the new strategy was being implemented throughout the country. The result was exactly as the modelers had predicted: The disease was now contained, and tens of thousands of cows and sheep were spared. Prime Minister Tony Blair was sufficiently impressed by this practical, real-time application of science that he asked that the existing program for generating scientific advice be refurbished. Learning from the example of the Japanese government, a U.K. Technology Foresight Programme had been launched in 1994 to identify future wealth-creating opportunities from science, engineering, and technology and to encourage closer interaction between scientists and industry. It operated with standing panels of experts, each looking at the future for a particular area of technology.

However, we needed a way to capture the interdisciplinary knowledge generated in universities and research institutions around the world, to relate this knowledge to risks and opportunities that might arise in the future, and to use it to provide fully evidence-based policy advice to the U.K. cabinet. Thus, in 2002, a new phase of Foresight was launched. The standing panels were scrapped, to be replaced by a fluid, rolling program of three or four projects at a time, each taking about 2 years to produce their reports and findings.

The first step in ensuring that politicians are ready to listen, and that scientists are able to speak, is to choose the right topics. To appear on the Foresight short list, a topic must represent either (i) some important current issue that science, technology, the social sciences, and economics could help address (for example, flood risk management) or (ii) a current aspect of science or technology that is likely to have wider potential in the future (for example, exploiting new aspects of the electromagnetic spectrum). The subject must be future-oriented; must not duplicate work taking place elsewhere; must have potential outcomes that can lead to specific actions; must be multidisciplinary; and, above all, must have commitment from the potential beneficiaries that they are eager to hear the results and act on them.

We achieve this last criterion through very wide consultation. The short list of topics is posted on the Foresight Web site (*3*) for comments, and in parallel, the Foresight team consults scientists, the private sector, and government departments. No Foresight project is even started until it is clear that there is wide support. Crucially, each project must be sponsored by a minister from a relevant government department.

When a topic is chosen, a group of stakeholders is set up to oversee the project. The Chief Scientific Adviser directs this team, which is composed of senior decision-makers and budget-holders from relevant government departments, research councils, industry, charities, and other professional bodies. The group is chaired by the sponsoring minister.

Meanwhile, the project team, made up of

U.K. Foresight Topics

Flood and Coastal Defence, 2004 (published as the "Future Flooding" report)
Sponsoring: Minister for the Environment and Agri-Environment, Department for Environment Food and Rural Affairs

Cognitive Systems, 2003
Sponsoring: Science Minister, Department of Trade and Industry

Exploiting the Electromagnetic Spectrum, 2004
Sponsoring: Minister for Energy, e-Commerce and Postal Services, Department of Trade and Industry

Cyber Trust and Crime Prevention, 2004
Sponsoring: Minister for Crime Reduction, Policing, Community Services and Counter Terrorism, Home Office

Brain Science, Addiction and Drugs, 2005
Sponsoring: Minister for Health, Department of Health

Intelligent Infrastructure Systems, 2006
Sponsoring: Minister for Transport, Department for Transport

The Detection and Identification of Infectious Diseases, 2006
Sponsoring: Minister for Sustainable Farming and Food, Department for Environment Food and Rural Affairs

Tackling Obesity: Future Choices, 2007
Sponsoring: Minister for Public Health, Department of Health

Sustainable Energy Management and the Built Environment, 2008
Sponsoring: Minister for Housing and Planning, Department of Communities and Local Government

Mental Capital and Wellbeing, 2008
Sponsoring: Minister for Lifelong Learning, Further and Higher Education, Department For Education and Skills.

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civil servants and up to six external leading experts, sets about inviting between 90 and 120 natural scientists, social scientists, and economists onto the project. These participants review the scientific literature extensively and look at relevant social and economic trends. They also undertake "horizon scanning," that is, they consider what developments lie in the near future through a series of workshops, seminars, brainstorming sessions, and other conversations with industry and relevant professional organizations. As well as bringing together the results of existing research, the projects can commission new research, for example, modeling studies that specifically address the problem at hand.

At the end, the team produces a set of clear, comprehensive, and comprehensible science reports. Often, we employ science writers to rewrite the reviews of the specialists so that they are accessible to all the interdisciplinary team members. In many cases, this has been vital to the success of the process. A range of techniques [such as scenario-planning or technology road-mapping (*4*, *5*)] are used to trace different possible futures according to varying social, political, and scientific drivers and to describe likely outcomes for alternative visions. The results are never simply extrapolations from the present day.

Another product of each project is an action plan that is widely circulated to all stakeholders and publicly available. The primary aim is that Foresight projects will influence both policy and funding decisions made by government. There is little point in producing scientific reports if nobody on the political side has committed themselves to listening. It also seeks to inform decision-making by business, charities, and funding bodies.

To determine whether the projects are having the desired impact, each project has a follow-up meeting a year after the results are published. This meeting, chaired by the lead minister, assesses how (or indeed whether) the project findings are being addressed. It either ensures that the project results are having an effective influence or, if not, determines why not. Depending on the project, there may be other follow-up activities to keep up the momentum, and the Prime Minister and Cabinet are kept appropriately informed.

Since 2002, seven projects have been completed, and three more are in the pipeline (see the table on page 1701). All have been influential. In particular, several projects have influenced key (and often otherwise science-lite) areas of policy, spending decisions, and research agendas.

One of the first projects to be undertaken, the Flood and Coastal Defence Project, investigated the potential threat to Britain from rising sea levels and changing rainfall patterns that could result from predicted changes in climate over the next 80 years. This was the first detailed regional study of climate change impacts conducted specifically to advise a government what actions to take. An important outcome was the realization that coasts are not the only places threatened. The predicted increases in rainfall intensities are likely to overwhelm the drainage and sewerage systems for British towns designed and built for the Victorian age, and the potential for floods even in inland towns and cities is thus very high.

Foresight programs can adopt highly imaginative approaches to problem-solving in addition to the conventional ones. In analyzing coastal vulnerabilities, we commissioned a computer game [inspired by Sim City and available as "FloodRanger" (*6*)] that demonstrates how strategies for a British coastal town could be tested against future weather superimposed on climate trends.

In 1998, the U.K. Government was spending about £300 million (U.S. $590 million) per year on managing these risks. Thanks in part to the Foresight program, we have managed to increase that amount to more than £500 million per year (U.S. $984 million). A further outcome was the realization that adaptation to climate change impacts is strongly dependent on international action in mitigating greenhouse gas emissions (*7*). Through an agreement between the U.K. and Chinese governments, the lessons learned over a 2½-year period by the British Flooding Team are being passed on to a Chinese team for a study of flood impacts from fluvial and coastal flooding in the Shanghai region.

Foresight programs can also have international impact. The G8 Summit Communiqué on the Fight Against Infectious Diseases (*8*) reflects several of the Foresight findings from its project on the Detection and Identification of Infectious Diseases. The project was unique in many ways. We engaged with about 400 scientists, including 50 each from Africa and China, and we addressed disease in plants, animals, and humans. The stakeholder board included members of the three international organizations in each of these fields, the Food and Agriculture Organization of the United Nations, the World Organization for Animal Health, and the World Health Organization. One conclusion, that about 80% of human infectious diseases derive from animals, reflected the need for more transdisciplinary work in these areas (*9*). Of critical importance, the project described the enormous potential power in controlling the spread of infectious diseases we would have if handheld disease-monitoring devices, built with the communication capability of the mobile phone, could be developed and widely distributed. As a result, the U.K. government's Department for Environment, Food, and Rural Affairs set up a technology program to develop such devices, which they have funded at £800,000 (U.S. $1,574,000).

The Foresight program still faces two challenges inherent to working in government. Ministers change departments, and enthusiastic support for a project and its action plans may not be sustained. Furthermore, Foresight projects are, by their very nature, interdisciplinary in focus, and the cross-cutting issues under study are often the responsibility of more than one government department. Historically, departments have been protective of their own responsibilities, and an enthusiastic minister sponsoring a project may not be able to get support from colleagues elsewhere. We are seeking to address these challenges.

Foresight projects go deep, which is why we have limited them to no more than four at any one time. To complement the program, in 2005 we set up a Horizon Scanning Centre whose responsibility is to analyze risks and opportunities that are already on the horizon. For instance, to address the changing landscape of trade with Asia, the center ran workshops in five Asian countries, asking participants to rate social, technological, environmental, and political issues and to prioritize what will be driving their trade in the near future. This information was distilled and presented to the U.K. government. Horizon-scanning projects are narrower and swifter than their Foresight cousins but can also feed back to Foresight by identifying topics worthy of more in-depth attention.

There is an adage that politicians use science the way a drunk uses a lamppost—more for support than for illumination. The lesson of the Foresight program is that the wisest decisions are made when science is at the very heart of policy: You can govern without the benefit of science, but you cannot govern well.

References

1. N. Ferguson, C. A. Donnelly, R. M. Anderson, *Science* **292**, 1155 (2001).
2. M. Keeling *et al.*, *Science* **294**, 813 (2001).
3. Foresight, www.foresight.gov.uk.
4. A. Curry, T. Hodgson, R. Kelnar, A. Wilson, *Foresight: Intelligent Infrastructure Futures: The Scenarios—Towards 2055* (Office of Science and Innovation, London, 2006).
5. I. Barker *et al.*, *Foresight: Infectious Diseases: Preparing for the Future—A Vision of Future Detection, Identification, and Monitoring Systems* (Office of Science and Innovation, London, 2006).
6. FloodRanger, www.discoversoftware.co/uk/FloodRanger.htm.
7. D. A. King, *Science* **303**, 177 (2004).
8. G8 Summit Communiqué on the Fight Against Infectious Diseases; http://en.g8russia.ru/docs/10.html
9. M. E. J. Woolhouse *et al.* *Science* **313**, 1392 (2006).

10.1126/science.1146051

GENETICS

A Breakthrough for Global Public Health

The *Aedes aegypti* genomic sequence provides new opportunities to understand the basic biology and evolution of the mosquito and to mitigate the impact of this disease vector on public health.

Dave D. Chadee, Pattamaporn Kittayapong, Amy C. Morrison, Walter J. Tabachnick

The reemergence of dengue fever and urban yellow fever in the Americas during the past 20 years demonstrates that mosquito-borne diseases are threats even in the 21st century. Globally, about 50 million to 100 million cases of dengue and about 500,000 cases of dengue hemorrhagic fever occur annually (*1*). Recently, an unprecedented chikungunya virus outbreak occurred in countries bordering the Indian Ocean, with ~250,000 cases and 205 deaths. The threat of mosquito-borne pathogens is very real, with dengue, yellow fever, and chikungunya viruses all being transmitted by the mosquito *Aedes aegypti*.

Yellow fever mosquito. The painting "The Conquerors of Yellow Fever" by Dean Cornwell (1939) shows an early yellow fever transmission trial in humans. (**Standing left to right**) Carlos Finlay (in dark suit), Aristedes Agramonte (holding hat), Jesse Lazear (applying cage with mosquito), and Walter Reed (in white uniform).

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On page 1718 of this issue, Nene *et al.* report the complete genome sequence of *Ae. aegypti* (*2*). This comes about 4 years after the complete genome sequence of *Anopheles gambiae*, the primary mosquito vector of malaria in Africa (*3*). It is also a little over 100 years since *Ae. aegypti* was shown to transmit yellow fever. As the blueprint for the vector's biology, the *Ae. aegypti* genome sequence is another major advance in the history of combating mosquito-borne disease. Mosquito-borne disease control is currently based on clinical management of patients and mosquito control because efficient vaccines are unavailable. The challenge ahead is to use genome sequence information to understand gene and protein functions and the causes of mosquito diversity that determine the role of *Ae. aegypti* in pathogen transmission (*4*).

The completed sequence should greatly facilitate the identification of *Ae. aegypti* genes and proteins that control a wide range of traits such as vector competence and capacity for pathogen transmission, life history, olfactory cues that affect behavior, host seeking, mating behavior, and insecticide resistance. The genome sequence should also help identify new DNA markers and allow DNA fingerprinting for ecological studies. Such tools are essential to characterize both individual mosquitoes and natural populations of *Ae. aegypti*. Genetic characterization of mosquito populations should reveal how pathogen transmission is influenced by gene flow, geographic isolation, and population dynamics and dispersal. Moreover, characterizing gene variation in natural populations will provide a basis for understanding the risk for *Ae. aegypti*–borne epidemics. For example, yellow fever has never been reported in Asia, despite the presence of dengue and *Ae. aegypti*. Such an epidemic would be a catastrophe.

Tools for genetically altering *Ae. aegypti* (or *An. gambiae*) can now be more easily adapted for creating mosquitoes that are pathogen-resistant (*5*). In combination with new information on the effects of genes on the mosquito phenotype, deploying such resistant mosquitoes should be possible. Endosymbiotic bacteria could also be genetically modified to introduce desirable genes into mosquito populations that reduce vector competence for a pathogen or reduce their survival (*6*), a strategy guided by a deeper understanding of *Ae. aegypti* biology through its genome sequence.

The *Ae. aegypti* subspecies, *Ae. aegypti aegypti* and *Ae. aegypti formosus*, differ in appearance, geographical distribution, behavior, genetic diversity and relatedness, and vector competence for yellow fever virus (*7*) and dengue virus (*8*). The completed genome sequence is from *Ae. aegypti aegypti* because it is widely distributed, is the primary vector, and likely evolved from *Ae. aegypti formosus* (*7*). But the relationship between the geographic distribution and genetic diversity of *Ae. aegypti* must be clarified to ensure that control strategies are used that are appropriate for the specific location. Genetic mapping studies have implicated many genes in *Ae. aegypti* vector competence for dengue virus (*8*). However, specific genes have not yet been identified; the *Ae. aegypti* genome sequence should help in this characterization. The *An. gambiae* genome sequence has been essential to identifying candidate genes that control susceptibility to malaria infection. This was accomplished through the use of RNA interference, a technique that "knocks down" the expression of specific gene targets in the organism (*9*).

Many important mosquito behaviors are regulated by the mosquito's olfactory system, and the genes relevant to this process can now be identified through sequence comparisons to olfactory genes from other insects (*10*). Similar approaches will identify candidate

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genes and proteins involved in *Ae. aegypti* mating that could provide new ways to control *Ae. aegypti* populations.

Numerous *Ae. aegypti* control strategies have been tried over the past 100 years. There have been success stories, such as yellow fever eradication in Havana at the beginning of the 20th century, through mosquito larva reduction. However, success in reducing dengue has been largely unsustainable (*11*), contributing to the resurgence of the disease in the Americas. Biocontrol agents such as larval predators have had only limited success (*12*). The failure to control *Ae. aegypti* in the Caribbean is due in part to insecticide resistance, a situation that is further exacerbated by the lack of new insecticides.

The basic *Ae. aegypti* "blueprint" is in hand, but as is often the case for blueprint instructions, "some assembly required" is an understated summary of the extent of the challenges ahead for ultimately translating the sequence into meaningful information about the mosquito's biology (*4*). Having the genome sequence is surely an important step toward better limiting the distribution and burden of diseases transmitted by *Ae. aegypti*.

References

1. World Health Organization, *Fact Sheet 117* (2002).
2. V. Nene *et al.*, *Science* **316**, 1718 (2007); published online 17 May 2007 (10.1126/science.1138878).
3. R. A. Holtz *et al.*, *Science* **298**, 129 (2002).
4. W. J. Tabachnick, *J. Med. Entomol.* **40**, 597 (2003).
5. A. A. James *et al.*, *Trends Parasitol.* **21**, 64 (2005).
6. S. P. Sinkins, *Insect Biochem. Mol. Biol.* **34**, 723 (2004).
7. W. J. Tabachnick, *Am. Entomol.* **37**, 14 (1991).
8. K. E. Bennett *et al.*, *Genetics* **170**, 185 (2005).
9. M. M. Riehle *et al.*, *Science* **312**, 577 (2006).
10. C. M. Morel *et al.*, *Science* **298**, 79 (2002).
11. R. W. Justice *et al.*, *Bioessays* **25**, 1011 (2003).
12. D. D. Chadee *et al.*, *Trop. Med. Int. Health* **10**, 748 (2005).

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CHEMISTRY

Resolving an Elusive Structure

Synchrotron data allow the determination of the structure of the ubiquitous mineral ferrihydrite.

R. Lee Penn

Minerals are natural and homogeneous substances with clearly defined chemical composition and crystalline structure. Yet ferrihydrite—an iron oxide with a composition commonly given as $Fe_5HO_8 \cdot 4H_2O$—is officially classified as a mineral (*1*), despite a lack of consensus regarding its crystal structure, homogeneity, and even composition. Ferrihydrite occurs only as nanoparticles, which causes substantial broadening of the maxima in x-ray diffraction patterns and hinders the use of traditional methods for structure determination.

On page 1726 if this issue, Michel *et al.* (*2*) present the analysis of total x-ray scattering data (*3*) collected at a synchrotron source and propose a new model for the atomic arrangement in ferrihydrite. The work represents a major leap forward in understanding this important mineral and demonstrates the power of their methodology for elucidating the structures of nanocrystalline materials.

Ferrihydrite is a natural material that is ubiquitous at and near Earth's surface (*4*). It is the first material that forms upon neutralization of acidic solutions of ferric ions and upon oxidation of solutions of ferrous ions. It is the typical precursor to the more stable iron oxide minerals such as hematite (α-Fe_2O_3) and goethite (α-FeOOH). Indeed, ferrihydrite nanoparticles can be used to produce needle-shaped goethite nanoparticles of controlled size (*5*). Ferrihydrite is also a common product of weathering of iron-bearing minerals and of microbial oxidation of ferrous ions (*6*).

Ferrihydrite can act as a strong sorbent for numerous natural and anthropogenic chemical species, including heavy metals (*7*) and arsenate (*8*), and can participate in redox reactions (*9*). Finally, ferrihydrite is antiferromagnetic up to a temperature of ~422 K (*10*). If the nanoparticles are large enough to form a thermally stable single magnetic domain, then ferrihydrite can contribute substantially to the detectable magnetic signal of natural sediments. Given the widespread occurrence of ferrihydrite in natural and engineered systems, the elucidation of its crystal structure will have far-reaching impact.

The properties of nanocrystalline materi-

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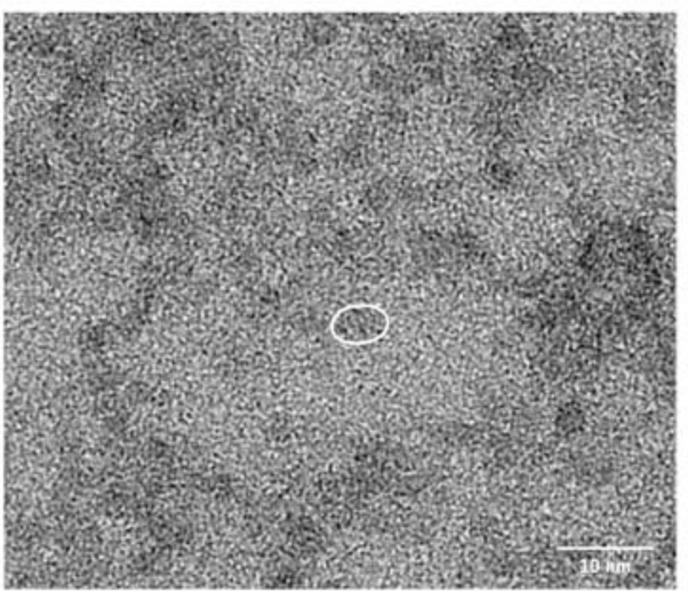

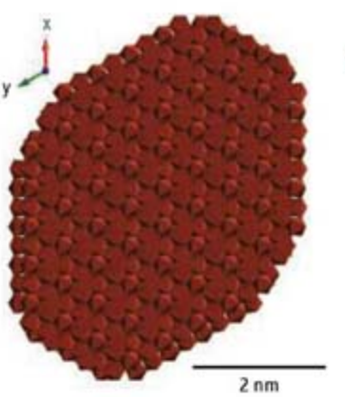

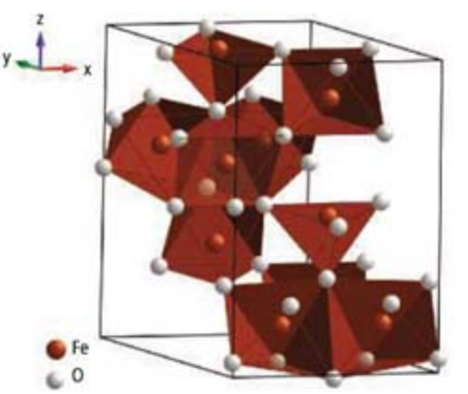

Resolving an elusive structure. Transmission electron micrograph (**left**) of nanocrystalline ferrihydrite prepared by controlled neutralization of a ferric nitrate solution with bicarbonate. Michel *et al.* have now uncovered the atomic structure of this material. On the basis of their proposed structure, the Crystalmaker program was used to create representations of a model nanoparticle (**middle**) and of the unit cell (**right**). The size of the former matches that of the ferrihydrite nanoparticle marked with the white circle in the left panel (*5*).

als—including iron oxides such as ferrihydrite—often depend on the size of the nanoparticles. Elucidation of these size-dependent effects is crucial for understanding the roles of iron oxides in environmental and geologic processes, such as the biogeochemical cycling of iron, weathering, and respiration of iron by microorganisms. Nanocrystalline materials can be characterized by many techniques, including electron microscopy (see the figure, left panel). However, none of these methods have been adequate for the structural determination of materials that are commonly referred to as "x-ray amorphous" (*11*)—meaning that the diffraction peaks are so broad as to make it nearly or totally impossible to solve their structure with laboratory-based x-ray diffraction instrumentation.

Michel *et al.* have now performed total elastic scattering experiments on ferrihydrite, which is x-ray amorphous, and have analyzed the data with the atomic pair distribution function (PDF) method. To obtain their structure, the authors calculated the PDF using structural models and then compared it to the PDF obtained from the experimental data. In addition to proposing a new model for the structure of ferrihydrite (see the figure, middle and right panels), the authors show that other recent structural models for ferrihydrite, including one that has gained a reasonable level of "acceptance" (*12*), produce a worse fit with the experimental data. This makes their proposed structure all the more convincing.

PDF analysis enables the extraction of structural information from powder diffraction data. This approach has for some time been the tool of choice for studying the atomic structure of liquids and glasses. The availability of focused, high-energy x-ray beams and of fast area detectors and advanced data treatment strategies has made it possible to apply this method to poorly crystalline and nanocrystalline materials. For example, Petkov *et al.* have used the approach to examine the atomic structures of vanadia xerogel (*13*) and of gold nanoparticles in water (*14*).

In a PDF analysis, the PDF is obtained by Fourier transformation of the total elastic scattering data. Use of a high-energy x-ray beam, available at third-generation synchrotron x-ray sources, is crucial, because the short wavelengths enable collection of diffraction data at much higher resolution than can be achieved in the laboratory.

The resulting PDF is a real-space representation of interatomic distances that includes both the short-range (1 to 5 Å) and intermediate- to long-range correlations (5 to potentially more than 100 Å) for all pairs of atoms in the structure. The sensitivity, resolution, and extended range of information allow real-space fitting of structural models for nanocrystalline and disordered materials, without the detrimental peak-broadening effects incurred during structure refinement in reciprocal space.

As the study by Michel *et al.* shows, the PDF method is a powerful tool for elucidating the structures of natural and synthetic nanoparticulate materials. It can also be used to study how atomic structure varies as a function of particle size and environment, a matter of crucial importance both for designing new nanomaterials and for understanding the properties of natural materials such as ferrihydrite.

References and Notes

1. Minerals are approved by the Commission on New Minerals and Mineral Names, International Mineralogical Association; see www.geo.vu.nl/users/ima-cnmmn/.
2. F. M. Michel *et al.*, *Science*, **316**, 1726 (2007); published online 24 May 2007 (10.1126/science.1142525).
3. In total x-ray scattering, both the Bragg (coherent) and elastic diffuse scattering are included. By way of comparison, only the Bragg component is included in the typical methods applied using laboratory-based diffractometers.
4. J. L. Jambor, J. E. Dutrizac, *Chem. Rev.* **98**, 2549 (1998).
5. R. L. Penn, J. Erbs, D. M. Gulliver, *J. Cryst. Growth* **293**, 1 (2006).
6. J. F. Banfield, S. A. Welch, H. Z. Zhang, T. T. Ebert, R. L. Penn, *Science* **289**, 751 (2000).
7. R. G. Ford, P. M. Bertsch, K. J. Farley, *Environ. Sci. Technol.* **31**, 2028 (1997).
8. K. P. Raven, A. Jain, R. H. Loeppert, *Environ. Sci. Technol.* **32**, 344 (1998).
9. D. Fortin, S. Langley, *Earth Sci. Rev.* **72**, 1 (2005).
10. T. S. Berquo, S. K. Banerjee, R. G. Ford, R. L. Penn, T. Pichler, *J. Geophys. Res.* **112**, B02102/1 (2007).
11. F. M. Michel *et al.*, *Chem. Mater.* **17**, 6246 (2005).
12. V. A. Drits, B. A. Sakharov, A. L. Salyn, A. Manceau, *Clay Miner.* **28**, 185 (1993).
13. V. Petkov *et al.*, *J. Am. Chem. Soc.* **124**, 10157 (2002).
14. V. Petkov *et al.*, *Phys. Rev. B* **72**, 195402 (2005).

15. I thank the University of Minnesota and NSF (grant MRI EAR-0320641) for funding the purchase of the Tecnai F30 used to collect the TEM image shown in the figure, and S. K. Banerjee and F. M. Michel for helpful and insightful comments.

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PHYSICS

Is There Glue in Cuprate Superconductors?

Philip W. Anderson

Many theories about electron pairing in cuprate superconductors may be on the wrong track.

More than 20 years after the discovery of cuprate superconductors, physicists do not agree on what mechanism causes the loss of electrical resistance at temperatures as high as 160 K (known as T_c, the transition temperature). They do agree that electron pairs are crucial because they can form a condensate that flows without resistance, but the interaction that causes the pairs to form is disputed.

For many years, papers have been appearing that discuss the high-T_c copper oxide superconductors in the same terms as the conventional metallic superconductors (such as mercury or lead). That is, some researchers assume that the high-T_c materials involve electron pairs bound together by the exchange of bosons (a fundamental class of particles, the other being fermions). In the ordinary superconducting metals, these exchanged particles are phonons (atomic lattice vibrations) that act like a bosonic "glue" to hold the electron pairs together. Many alternatives have been proposed for this bosonic glue (*1*–*9*). This mythology is popular among science journalists, who dramatize both the element of competition and the search for The Secret.

I argue here that this need for a bosonic glue is folklore rather than the result of scientific logic. It comes from the inappropriate assumption that superconductivity in these materials is described by a mathematical framework called the Eliashberg formalism (*10*), which is an extension of the original ideas of Bardeen, Cooper, and Schrieffer. In the 1960s, Morel and I (*11*) and Schrieffer *et al.* (*12*) adapted this formalism to calculate properties of the conventional superconductors, but it is valid only to describe the particular mechanism that explains these superconductors.

Electrons only interact, to a very good approximation, via the Coulomb interaction. This is the elementary electrical force that causes two negative charges to repel each other. So how can this repulsion between electrons be eliminated in favor of electron pair

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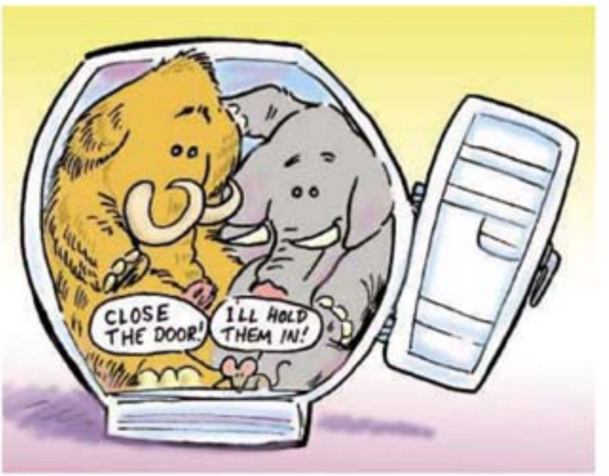
binding? The possibilities are either "dynamic screening" or a mechanism suggested by Pitaevskii (*13*) and by Brueckner *et al.* (*14*) of putting the electron pairs in an anisotropic wave function (such as a d-wave), which vanishes at the repulsive core of the Coulomb interaction. In either case, the paired electrons are seldom or never in the same place at the same time. Dynamic screening is found in conventional superconductors, and the anisotropic wave functions are found in the high-T_c cuprates and many other unconventional superconductors.

In the case of dynamic screening, the Coulomb interaction e^2/r (where e is the electron charge and r is the distance between charges) is suppressed by the dielectric constant of other electrons and ions. The plasma of other electrons damps away the long-range $1/r$ behavior and leaves a screened core, $e^2 \exp(-\kappa r)/r$ (where κ is the screening constant), that acts instantaneously, for practical purposes, and is still very repulsive. By taking the Fourier transform of the interaction in both space and time, we obtain a potential energy V, which is a function of frequency ω and wavenumber q; the screened Coulombic core, for instance, transforms to $V_s = e^2/(q^2 + \kappa^2)$ and is independent of frequency. This interaction must then be screened by the dielectric constant ε_{ph} because of polarization of the phonons, leading to a final expression $V = e^2/[(q^2+\kappa^2)\varepsilon_{ph}(q, \omega)]$. This dielectric constant is different from 1 only near the lower frequencies of the phonons. It screens out much of the Coulomb repulsion, but "overscreening" doesn't happen: When we get to the very low frequency of the energy gap, V is still repulsive.

Instead of accounting for the interaction as a whole, the Eliashberg picture treats only the phonon contribution formally, replacing the high-frequency part of the potential with a single parameter. But the dielectric description more completely clarifies the physics, and in particular it brings out the limitations on the magnitude of the interaction. That is, it makes clear that the attractive phonon interaction, characterized by a dimensionless parameter λ, may never be much bigger, and is normally smaller, than the screened Coulomb repulsion, characterized by a parameter μ (*11*). The net interaction is thus repulsive even in the phonon case.

How then do we ever get bound pairs, if the interaction is never attractive? This occurs because of the difference in frequency scales of the two pieces of the interaction. The two electrons about to form a pair can avoid each other (and thus weaken the repulsion) by modifying the high-energy parts of their relative wave function; thus, at the low energies of phonons, the effective repulsive potential becomes weaker. In language that became familiar in the days of quantum electrodynamics, we can say that the repulsive parameter μ can be renormalized to an effective potential or "pseudopotential" μ^*. The effective interaction is then $-(\lambda - \mu^*)$, which is less than zero, hence attractive and pair-forming. One could say that superconductivity results from the bosonic interaction via phonons; but it is equally valid to say instead that it results from the renormalization that gives us the pseudopotential μ^* rather than μ. This does not appear in an Eliashberg analysis; it is just the type of correction ignored in this analysis.



"We have a mammoth and an elephant in our refrigerator—do we care much if there is also a mouse?"

The above is an instructive example to show that the Eliashberg theory is by no means a formalism that universally demonstrates the nature of the pairing interaction; it is merely a convenient effective theory of any portion of the interaction that comes from low-frequency bosons. There is no reason to believe that this framework is appropriate to describe a system where the pairing depends on entirely different physics.

Such a system occurs in the cuprate superconductors. The key difference from the classic superconductors, which are polyelectronic metals, is that the relevant electrons are in a single antibonding band that may be built up from linear sums of local functions of x^2-y^2 symmetry, with a band energy that is bounded at both high and low energies. In such a band the ladder-sum renormalization of the local Coulomb repulsion, leading to the pseudopotential μ^*, simply does not work, because the interaction is bigger than the energy width of the band. This is why the Hubbard repulsion U between two electrons on the same atom (which is the number we use in this case to characterize the repulsion) is all-important in this band. This fact is confirmed by the Mott insulator character of the undoped cuprate, which is an antiferromagnetic insulator with a gap of 2 eV, giving us a lower limit for U.

But effects of U are not at all confined to the cuprates with small doping. In low-energy wave functions of the doped system, the electrons simply avoid being on the same site. As a consequence, the electrons scatter each other very strongly (*15*) and most of the broad structure in the electrons' energy distribution functions (as measured by angle-resolved photoelectron spectroscopy) is caused by U. This structure may naïvely be described by coupling to a broad spectrum of bosonic modes (*4*), but they don't help with pair binding. U is a simple particle-particle interaction with no low-frequency dynamics.

A second consequence of U is the appearance of a large antiferromagnetic exchange coupling J, which attracts electrons of opposite spins to be on neighboring sites. This is the result of states of very high energy, and the corresponding interaction has only high-frequency dynamics, so it is unrelated to a "glue." There is a common misapprehension that it has some relation to low-frequency spin fluctuations (*16*, *17*), but that is incorrect, as low-frequency spin interactions between band electrons are rigorously ferromagnetic in sign. One can hardly deny the presence of J given that it has so many experimental consequences.

In order to avoid the repulsive potential these systems are described by the alternative Pitaevskii-Brueckner-Anderson scheme with pairing orthogonal to the local potential. Two such pairings exist, d-wave and "extended s-wave," but only one appears as a superconducting gap; the extended s-wave is unsuitable for a gap and acts as a conventional self-energy (*18*). The specific feature of the low-dimensional square copper lattice that is uniquely favorable to high T_c is the existence of the two independent channels for pairing (*18*). Because of the large magnitude of J, the pairing can be very strong, but only a fraction of this pairing energy shows up as a superconducting T_c, for various rather complicated but well-understood reasons.

The crucial point is that there are two very strong interactions, U (>2 eV) and J (~0.12 eV), that we know are present in the cuprates, both a priori and because of incontrovertible experimental evidence. Neither is properly described by a bosonic glue, and between the two it is easy to account for the

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existence of antiferromagnetism, d-wave superconductivity, and many other phenomena of high-T_c superconductivity. Whether any additional "glue" exists is of lesser interest. We have a mammoth and an elephant in our refrigerator—do we care much if there is also a mouse?

References and Notes

1. P. Paci, C. Grimaldi, L. Pietronero, http://arxiv.org/abs/cond-mat/0005217 (2000).
2. X. J. Zhou, T. Cuk, T. Devereaux, N. Nagaosa, Z.-X. Shen, http://arxiv.org/abs/cond-mat/0604284 (2006).
3. E. Demler, S. C. Zhang, *Nature* **396**, 733 (1998).
4. M. R. Norman, A. V. Chubukov, *Phys. Rev. B* **73**, 140501 (2006).
5. J. Hwang *et al.*, http://arxiv.org/abs/cond-mat/0607653 (2006).
6. J. C. Davis *et al.*, *Nature* **452**, 546 (2006).
7. A. J. Millis, H. Monien, D. Pines, *Phys. Rev. B* **42**, 167 (1990).
8. P. Monthoux, A. V. Balatsky, D. Pines, *Phys. Rev. Lett.* **67**, 3448 (1989).
9. The above papers (*1*–*8*) are typical of many that have appeared over the years; I have selected them mostly on the basis of recent interest.
10. D. J. Scalapino, J. M. Rowell, in *Superconductivity*, R. D. Parks, Ed. (Dekker, New York, 1969), pp. 449–611.
11. P. Morel, P. W. Anderson, *Phys. Rev.* **125**, 1263 (1962).
12. J. R. Schrieffer, D. Scalapino, J. Wilkins, *Phys. Rev. Lett.* **10**, 336 (1963).
13. L. P. Pitaevskii, *Sov. Phys. JETP* **10**, 1267 (1960).
14. K. A. Brueckner *et al.*, *Phys. Rev.* **118**, 1442 (1960).
15. P. W. Anderson, *Nat. Phys.* **2**, 626 (2006).
16. D. J. Scalapino, E. Loh Jr., J. Hirsch, *Phys. Rev. B* **34**, 8190 (1986).
17. T. Moriya, *J. Phys. Soc. Jpn.* **59**, 2905 (1990).
18. P. W. Anderson, *Phys. Rev. Lett.* **96**, 017001 (2006).

19. I acknowledge discussions with J. C. Davis, T. Timusk, L. Pietronero, N. P. Ong, and A. Yazdani.

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CHEMISTRY

Making Energy Count

Experiments reveal the subtle roles of vibrational and translational energy in reactions of polyatomic molecules.

F. Fleming Crim

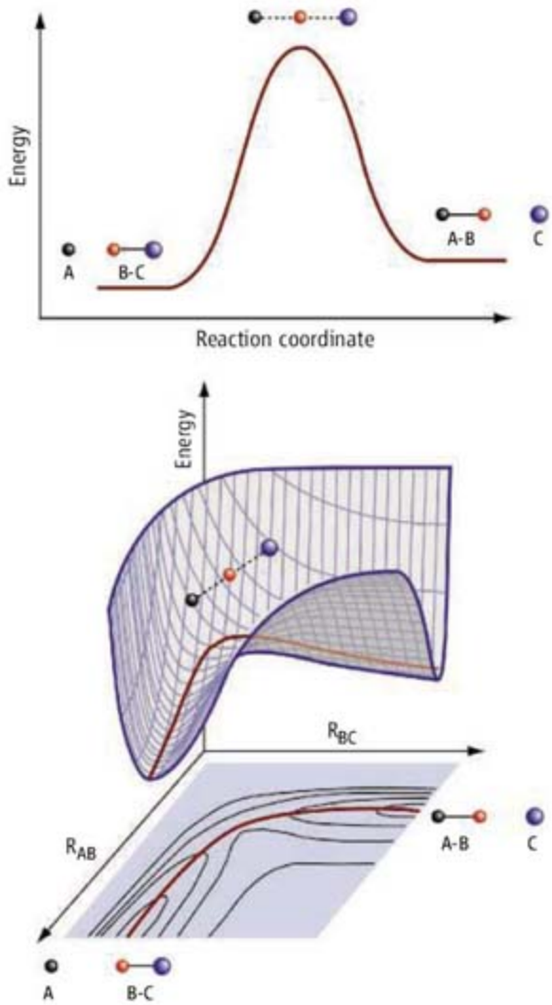
Energy influences the rates of chemical reactions dramatically (*1*). Simply heating a reaction mixture deposits energy indiscriminately in internal and translational motion, but more specific excitation can change the course of a reaction. The challenge is to distinguish the effect of these different types of energy.

For reaction of atoms with diatomic molecules, knowledge of the geometry of the system at the energy barrier for the reaction is sufficient to predict the relative efficacy of vibrational and translational energy (*2*). However, few experimental studies have investigated the effectiveness of different types of energy in more complicated molecules. On page 1723 of this issue, Yan *et al.* (*3*) explore the role of vibrational and translational energy in a prototypical reaction of a polyatomic molecule.

Chemical kinetics centers on the concepts of a transition state (the geometry through which reactants pass as they rearrange their bonds to become products) and of a reaction coordinate (the minimum-energy path along which the atoms move to reach and pass through the transition state). To react, molecules must have sufficient energy to reach the transition state, and this energy must reside in motions that carry the system through the transition state.

Two-dimensional energy plots along the reaction coordinate for the reaction of A with BC (see the figure, top panel) convey little information about the motions involved. Varying the angle between A and BC produces a family of three-dimensional surfaces, one for each angle, that form a four-dimensional hypersurface. The best we can do in three dimensions is a "cut" through this hypersurface for a single angle (see the figure, bottom panel).

The situation is more complex for polyatomic molecules. The reaction hypersurface has more dimensions, and there are often several transition states leading to different products. Perhaps most important, there are many more vibrations in the reactant. Nevertheless, the concepts of a reaction coordinate and transition state remain useful for understanding the role of different types of energy in these reactions.



Reaction dynamics. In the reaction studied by Yan *et al.*, the CD_3 group is the "atom" C, and Cl and H are the atoms A and B, respectively. (**Top**) Two-dimensional view of the energy along the reaction coordinate for the reaction A + BC→AB + C. The highest-energy point is the transition state. (**Bottom**) Three-dimensional view of the energy surface for the reaction shown at the top, along with a contour plot of the surface. The geometry of A-B-C is linear. The coordinates are the length of the "new" bond, R_{AB}, and the length of the "old" bond, R_{BC}. The red line is the reaction coordinate.

Yan *et al.* study the reaction of Cl with CHD_3, which has two available paths: One breaks the C-H bond to form HCl + CD_3, and the other breaks the C-D bond to form DCl + CHD_2. Stretching of the reactant bond appears to be part of the motion along the reaction coordinate. Therefore, an intuitively appealing means of accelerating a reaction is to place vibrational energy in the bond that is to be broken. This approach is a proven means of preferentially cleaving the vibrationally excited bond in the reactions of Cl with partially deuterated methanes (*4*, *5*). These experiments also show that vibrational excitation

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increases the rate of the reaction over that for room-temperature molecules, but they do not explore how vibrational energy compares to translational energy in promoting the reaction.

Addressing this question requires controlled deposition of energy in either translation or vibration. Yan *et al.* have done so by combining molecular beam scattering (in which they control the translational energy precisely) with vibrational excitation of the reactants and angularly resolved detection of the products. Studying the pathway that cleaves the C-H bond, $Cl + CHD_3 \rightarrow HCl + CD_3$, they find that energy in the C-H stretching vibration or in translation increases the rate of the reaction by about the same amount. Energy in the bending vibration, deposited by heating the CHD_3 molecules in the molecular beam source, is slightly more effective than translation.

These observations point to the subtleties of reactions of polyatomic molecules. The simplest extension of the correlations developed for three-atom systems is to consider only the bond that breaks, treating the CD_3 group as a single atom in the reaction of Cl with H-CD_3. The location of the transition state for $Cl + CHD_3$ suggests that vibrations should promote the reaction more effectively than translations, contrary to the experimental observation (*3*). Even in a reaction of a polyatomic molecule as small as methane, the structure of the nonreacting part of the molecule is potentially important. For example, the initially excited vibration can evolve into a different set of motions as the reactive atom approaches, even while preserving its excitation (*6*, *7*).

The angular distributions measured by Yan *et al.* suggest that there is a delicate balance of such changes. Although stretching vibration and translation enhance the reaction to a comparable extent, the former produces vibrationally excited HCl products scattered forward in a narrow cone of angles, whereas the latter produces HCl in its vibrational ground state, scattered into a broad range of angles. Thus, vibrational and translational energy both accelerate the reaction, but do not lead to the same outcome. The differences can be subtle, as in the scattering direction of the products, or more obvious, as in the identity of the bond that breaks. Knowing the role of different kinds of energy allows better control over the outcome of a reaction and provides insights into the reactions of excited molecules in high-energy environments such as plasmas, combustion mixtures; and the atmosphere.

Simple pictures based on the idea that only a few motions are important in the reaction coordinate should be useful even for complicated reactions. As Yan *et al.* show, incisive experiments and calculations on prototypical systems can refine and guide the development of widely applicable and transferable models that identify those motions and predict their behavior.

References

1. I. Rombauer, M. R. Becker, *The Joy of Cooking* (Bobbs-Merrill, Indianapolis, IN, 1973).
2. J. C. Polanyi, *Acc. Chem. Res.* **5**, 161 (1972).
3. S. Yan, Y.-T. Wu, B. Zhang, X.-F. Yue, K. Liu, *Science* **316**, 1723 (2007).
4. S. Yoon, R. J. Holiday, F. F. Crim, *J. Chem. Phys.* **119**, 4755 (2003).
5. H. A. Bechtel, Z. H. Kim, J. P. Camden, R. N. Zare, *J. Chem. Phys.* **120**, 791 (2004).
6. S. Yoon, R. J. Holiday, E. L. Sibert, F. F. Crim, *J. Chem. Phys.* **119**, 9568 (2003).
7. L. Halonen, S.L. Bernasek, D. J. Nesbitt, *J. Chem. Phys.* **115**, 5611 (2001).

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CLIMATE CHANGE

Reassessing Carbon Sinks

Carbon dioxide is taken up more by the Southern Ocean, but less by tropical land areas, than previously thought.

David F. Baker

About 10 petagrams of carbon (Pg C) are released to the atmosphere as carbon dioxide (CO_2) each year by fossil fuel burning and deforestation. Less than half of this carbon stays in the atmosphere; the rest is taken up by the oceans and the terrestrial biosphere. The anthropogenic input has nearly tripled over the past 50 years, and the uptake has grown proportionally. Where does this uptake occur, and will it continue to grow? These questions must be answered if we are to be able to predict future CO_2 concentrations and the resulting climate change.

Atmospheric transport inversions can divide the global CO_2 uptake into regional sources and sinks. Two studies in this issue use such inversion results to question our current view of the carbon cycle. Le Quéré *et al.* on page 1735 of this issue (*1*) argue that the Southern Ocean sink has stopped growing, and Stephens *et al.* on page 1732 of this issue (*2*) suggest that the tropical land regions are a substantial CO_2 sink.

The Southern Ocean (south of 45°S) has long been thought to be a large and growing sink of fossil CO_2. The fierce circumpolar winds there mix the oceans to great depths, keeping surface CO_2 concentrations close to the slowly changing deep-ocean values. With atmospheric CO_2 rising and surface CO_2 roughly constant, the uptake of CO_2 in the Southern Ocean should be increasing, all else being equal. Le Quéré *et al.* looked in the atmosphere for the signature of this growing uptake but instead found no significant trend in the Southern Ocean sink since 1981. Using an ocean carbon model, Le Quéré *et al.* show that changes in the circumpolar winds are probably responsible for the stagnant trend. When they run their model with winds that do not vary from year to year, the sink grows steadily. But when they use actual historical winds, the uptake stays relatively flat. Further model runs show that the change in uptake mostly results from the impact of the winds on ocean mixing and upwelling.

The authors suggest that a recent trend toward more positive values of the Southern Annular Mode (SAM) may be driving these changes in upwelling. This possibility was first suggested by Wetzel *et al.* (*3*) and is explored in more detail by a recent modeling study (*4*). When the SAM is in a more positive phase, the circumpolar winds speed up. The effect this has on the Southern Ocean flux may be divided into two terms: the impact on the natural CO_2 fluxes that have been acting for millennia, and the impact on the anthropogenic perturbation to these fluxes, caused by rising atmospheric CO_2 concentrations and directed always into the ocean.

Stronger winds increase the anthropogenic uptake by increasing air-sea gas exchange and by mixing the carbon deeper into the ocean. However, they also increase the natural outgassing of CO_2 that prevailed across much of the Southern Ocean in preindustrial times. In the natural cycle, deep-ocean CO_2 levels are kept high by the biological pump (the transport of carbon from the surface to the deep ocean via sinking material derived from

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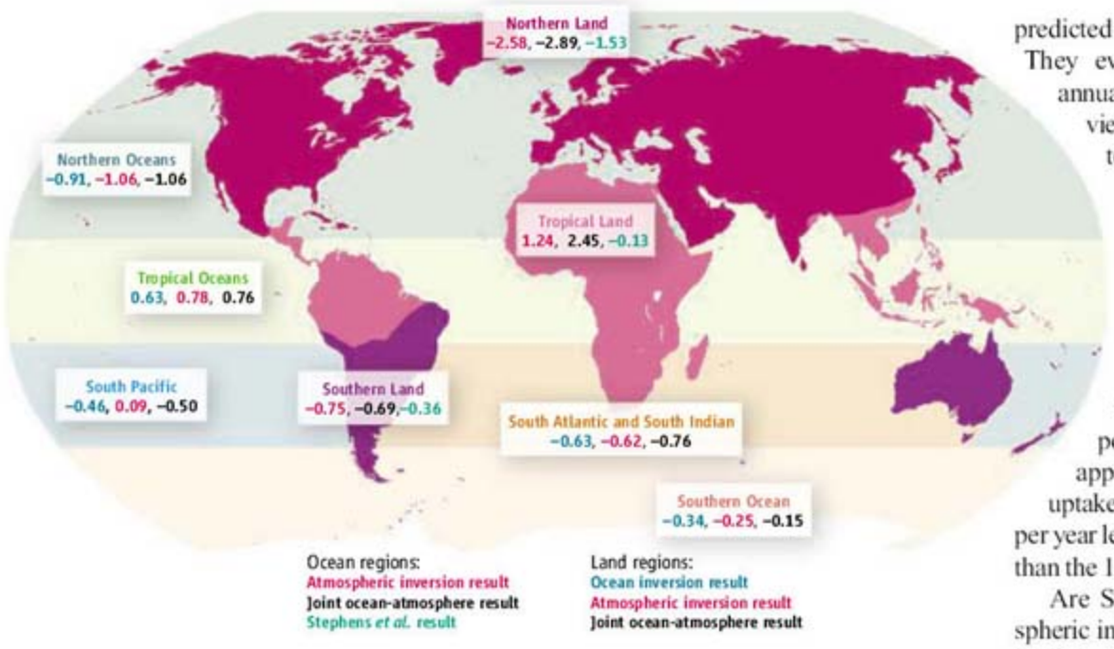

Regional CO_2 flux estimates. Three multimodel inverse estimates for the 1990s are given: the subsurface ocean inversions of Mikaloff Fletcher *et al.* (*5*, *6*) corrected for river fluxes (*11*) (blue); the TransCom3 interannual atmospheric inversion of Baker *et al.* (*9*) (red); and the joint ocean-atmosphere inversion of Jacobson *et al.* (*10*) (black). The oceanic and atmospheric inversions agree to within 1.5 Pg C per year for most ocean regions, including the Southern Ocean, for which the natural and anthropogenic fluxes mostly cancel, leaving a small net uptake (see the report by LeQuéré *et al.*). The land flux estimates for the three atmospheric models from Stephens *et al* (*2*) that best match observed vertical CO_2 profiles are also given (green). These suggest that transport model errors are causing overly large northern land uptake and tropical land release in the atmospheric and joint ocean-atmosphere inversion results. Fluxes are given in Pg C per year. Negative values indicate uptake from the atmosphere.

plankton). The circumpolar winds act as a large Ekman pump, driving these CO_2-rich deep waters to the surface, where they become supersaturated and release CO_2 to the atmosphere. In the model of Le Quéré *et al.* the increase in the upwelling-driven natural outgassing is large enough to cancel out the increasing anthropogenic sink.

The same sort of transport inversions used to interpret atmospheric CO_2 concentrations have recently been used to infer air-sea CO_2 fluxes from subsurface ocean carbon data. Mikaloff Fletcher *et al.* (*5*, *6*) have found that in preindustrial times, the Southern Ocean south of 44°S was a source of 0.4 Pg C per year but that it has become a net sink of 0.34 Pg C per year in recent decades as a result of the increasing uptake of anthropogenic CO_2. If Le Quéré *et al.* are right, the Southern Ocean sink stopped increasing by 1981, but it is still taking up CO_2 overall.

Will the Southern Ocean sink decrease and perhaps shift back to outgassing? If the SAM is indeed responsible for the observed slowdown, perhaps not. The SAM trend is thought to be mainly due to stratospheric ozone depletion, with only a smaller contribution from global warming (*7*). As the Antarctic ozone hole recovers, the Southern Ocean sink may well grow anew.

Whether or not the sink is still growing, the ocean inversions give a much lower value for the Southern Ocean uptake flux than did previous estimates based on surface ocean CO_2 measurements (*8*). The new value is closer to that given by recent atmospheric inversions (*9*). In fact, the oceanic and atmospheric CO_2 inversions have arrived at consistent estimates of decadal-scale uptake for almost all regions of the ocean (see the figure). This is heartening, given their independent data sources and errors. Where they differ, for example, in the South Pacific between 15°S and 45°S, the ocean inversions should be trusted more, given their greater spatial data coverage.

If the ocean fluxes in the atmospheric inversions are forced toward the values given by the ocean inversions, then the land regions are pushed toward strong uptake in the extra-tropical north, balanced by greater CO_2 release from the tropical land (as in the joint ocean-atmosphere inversion shown in the figure) (*10*). However, there has been little observational evidence to support the idea that more than 2 Pg C per year is being taken up over the northern continents. Stephens *et al.* argue that the northern land uptake is in fact not this large and that vertical mixing errors in the atmospheric transport models are biasing the inversion results.

Stephens *et al.* compare vertical profiles of CO_2 measured routinely by aircraft at 12 sites around the globe to the corresponding profiles predicted by 12 atmospheric transport models. They evaluate which models match the annual mean vertical profiles best. In their view, the models best satisfying this criterion have the most realistic vertical mixing and boundary layer thickness, produce the most accurate distributions of CO_2 when forced with fossil fuel, land biosphere, and ocean fluxes, and therefore give the most accurate flux estimates when used in atmospheric inversions. The three "best" transport models identified with this approach give ~1 Pg C per year less uptake by the northern land and ~1.7 Pg C per year less CO_2 release from the tropical land than the 12-model mean for 1992 to 1996.

Are Stephens *et al.* right that the atmospheric inversions should be corrected by this much—shifting over 1 Pg C/year of uptake from the northern to the tropical land regions? Is most of the tropical deforestation flux really compensated for by uptake elsewhere in the tropics? There are few measurements over the tropical land regions to help answer these questions, and convection-driven vertical motions ensure that those that do exist are sensitive to fluxes only in their immediate vicinity. NASA's Orbital Carbon Observatory (OCO) and the Japanese space agency's Greenhouse Gases Observing Satellite (GOSAT) may soon help. If these satellites can find holes in the persistent clouds over the tropical forests, their column-averaged data should discern the effects of convectively lofted surface fluxes much better than the current in situ measurements. Their lower sensitivity to vertical mixing should improve flux estimates over the northern land regions, too. They may be less useful in clarifying the Southern Ocean sink, however, because of persistently cloudy conditions there.

References

1. C. Le Quéré *et al.*, *Science*, **316**, 1735 (2007).
2. B. B. Stephens *et al.*, *Science* **316**, 1732 (2007).
3. P. Wetzel, A. Winguth, E. Maier-Reimer, *Glob. Biogeochem. Cycles* **19**, GB2005 (2005).
4. A. Lenton, R. J. Matear, *Glob. Biogeochem. Cycles* **21**, GB2016 (2007).
5. S. E. Mikaloff Fletcher *et al.*, *Glob. Biogeochem. Cycles* **20**, GB2002 (2006).
6. S. E. Mikaloff Fletcher *et al.*, *Glob. Biogeochem. Cycles* **21**, GB1010 (2007).
7. J. M. Arblaster, G. A. Meehl, *J. Climate* **19**, 2896 (2006).
8. T. Takahashi *et al.*, in *Proceedings of the 2nd International Symposium: CO_2 in the Oceans*, Y. Nojiri, Ed. (National Institute for Environmental Studies, Environment Agency of Japan, 1999), pp. 9–14.
9. D. F. Baker *et al.*, *Glob. Biogeochem. Cycles* **20**, GB1002 (2006).
10. A. R. Jacobson *et al.*, *Glob. Biogeochem. Cycles* **21**, GB1020 (2007).
11. N. Gruber *et al.*, *7th International Carbon Dioxide Conference* (2005).

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CELL SIGNALING

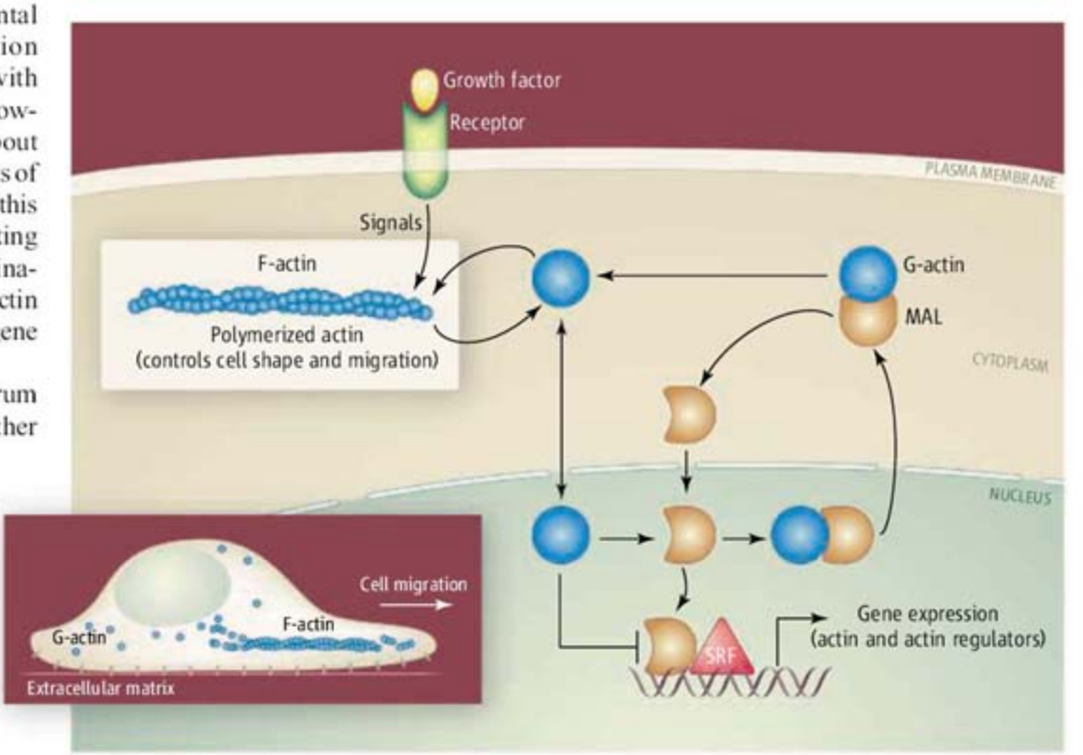
Nuclear Actin as Choreographer of Cell Morphology and Transcription

Changes in the cell's cytoskeleton affect gene expression through a signaling circuit that involves regulation of cofactor transport and function by actin in the nucleus.

Jiang I. Wu and Gerald R. Crabtree

Cellular responses to environmental stimuli often require coordination of rapid changes in cell shape with reprogramming of gene expression. However, relatively little has been known about how these essential events in different parts of the cell are harmonized. On page 1749 of this issue (*1*), Vartiainen *et al.* reveal an exciting new mechanism underlying this coordination, involving interactions between the actin cytoskeleton and a protein that regulates gene expression, called MAL.

Many mammalian cells respond to serum (which contains growth factors and other stimuli) by changing their morphology and activating gene expression through the serum response factor. The target genes of this transcription factor include those involved in cell growth, proliferation, and differentiation, as well as genes that control the actin cytoskeleton. Remarkably, serum response factor activity mirrors the state of cellular actin; a decrease in monomeric actin (G-actin) is both necessary and sufficient for serum response factor to activate gene expression (*2*). But how are changes in the amount of cellular G-actin communicated to the nucleus?



An actin circuit. Filamentous actin (F-actin) and monomeric actin (G-actin) pools are regulated by various signals triggered by growth factors and other stimuli present in serum. Signals that enhance actin polymerization cause MAL to move into the nucleus. Vartiainen *et al.* show that MAL is regulated by nuclear G-actin at multiple steps, as shown. SRF, serum response factor.

G-actin has dual residence in a cell. In the cytoplasm, it participates in a dynamic process of polymerization and depolymerization, generating actin filaments (F-actin) that can cause a cell's shape to change (see the figure). But G-actin also shuttles into and out of the nucleus, where it is thought to regulate chromatin structure and transcription. Prior to serum stimulation, MAL—a myocardin family transcriptional coactivator for serum response factor (*2*)—resides in the cytoplasm, where it interacts with G-actin. However, upon serum stimulation, F-actin forms to produce stress fibers in the cytoplasm, and G-actin levels decrease correspondingly. Sensing depletion of the G-actin pool, MAL dissociates from G-actin, and Vartiainen *et al.* show that this causes the already high basal rate of MAL import into the nucleus to increase.

In addition to a role in nuclear import, Vartiainen *et al.* demonstrate surprising roles for G-actin in regulating the export of MAL from the nucleus and in controlling the activation of serum response factor. MAL also binds to G-actin in the nucleus, and Vartiainen *et al.* show that this association is required for MAL to exit the nucleus. Thus, one would predict that after serum stimulation, a reduction of cytoplasmic G-actin (the cost of making F-actin) causes MAL to accumulate in the nucleus, thereby increasing MAL-dependent transcription. This indeed appears to be the case, but the explanation is more complex.

The authors show that nuclear accumulation alone is not enough for MAL to activate the expression of target genes by serum response factor. When MAL is prevented from exiting the nucleus (for example, by treating cells with the drug leptomycin B, which inhibits the cell's nuclear export machinery), the complex of MAL-serum response factor is transcriptionally inactive, even though it is poised on the promoters of target genes (shown by chromatin immunoprecipitation). Repression of serum response factor–dependent transcription is relieved by serum-induced actin polymerization in the cytoplasm, which depletes cytoplasmic G-actin. Because G-actin shuttles between the nucleus and the cytoplasm, events that promote actin polymerization in the cytoplasm also deplete nuclear G-actin (see the figure). Vartiainen *et al.* show that depletion of nuclear G-actin derepresses the expression of genes that require MAL for transcription by both reducing the rate of nuclear export of MAL and restoring MAL's ability to activate transcription after binding to target genes. This raises the question of how transcription by serum response factor is blocked by G-actin interaction with MAL in the nucleus.

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One possible explanation is that G-actin binding to MAL in the nucleus does not permit assembly of an effective transcription complex. Serum response factor lies at the nexus of two major signaling pathways that control the expression of different genes. One pathway involves the signaling enzyme MAP kinase and the activation of transcription coactivators of the ternary complex factor family (*3*). The other pathway relies on the myocardin family of coactivators (to which MAL belongs) (*4*). Because both families of coactivators bind to the same region of serum response factor, the G-actin–MAL complex may have reduced affinity for serum response factor. Another possible explanation relates to chromatin regulation by G-actin. In yeast and mammalian cells, G-actin is present in a number of protein complexes that remodel chromatin (*5*), enhancing adenosine triphosphatase activity of these complexes (*6*). It has been estimated that 10% of total nuclear G-actin is associated with SWI/SNF-like BAF chromatin-remodeling complexes. G-actin in these complexes may interact with MAL, physically linking MAL function to that of the remodeling complexes (inhibiting transcription), presumably by forming chromatin structures that repress gene expression. However, MAL has not been found associated with chromatin-remodeling complexes, making this explanation less attractive. A more likely explanation of how transcription by serum response factor is blocked by the MAL–G-actin complex is that G-actin simply interferes with MAL association with components of the general transcription apparatus, thus preventing serum response factor from activating transcription.

Skeptics might argue that many mechanisms elaborated in cultured cells may only be true of the cell line, with its specific chromosomal breakages, DNA methylation patterns, and thereby altered genetic circuits. This is almost certainly not the case with the work by Vartiainen *et al.* Although the authors used fibroblast cell lines, aspects of their mechanism are supported by rigorous genetic studies in mice. For example, deletion of the *serum response factor* gene in mice leads to death of embryos at gastrulation (*7*), when both transcription and actin-induced cell movement are essential. Conditional deletion of *serum response factor* in the murine nervous system produces specific defects in neurite outgrowth and neuron migration that are linked to reduced expression of actin and its regulators (*8, 9*). Finally, mice genetically engineered to lack *MAL* have defects in myoepithelial cell differentiation (*10*).

Cell biologists have long thought of actin regulation in the context of controlling cell morphology and movement. However, if confirmed by additional genetic studies (for example, analysis of mice with mutations in MAL that block its interaction with actin), the work by Vartiainen *et al*. elucidates how actin choreographs the regulation of morphology and transcription. Such a coordinated genetic circuitry must underlie such diverse events as early embryonic development, neuron migration, blood vessel formation, and lymphocyte signaling.

References

1. M. K. Vartiainen, S. Guettler, B. Larijani, R. Treisman, *Science* **316**, 1749 (2007).
2. F. Miralles, G. Posern, A. I. Zaromytidou, R. Treisman, *Cell* **113**, 329 (2003).
3. R. Treisman, *Curr. Opin. Genet. Dev.* **4**, 96 (1994).
4. G. C. Pipes, E. E. Creemers, E. N. Olson, *Genes Dev.* **20**, 1545 (2006).
5. I. A. Olave, S. L. Reck-Peterson, G. R. Crabtree, *Annu. Rev. Biochem.* **71**, 755 (2002).
6. K. Zhao *et al.*, *Cell* **95**, 625 (1998).
7. S. Arsenian, B. Weinhold, M. Oelgeschlager, U. Ruther, A. Nordheim, *EMBO J.* **17**, 6289 (1998).
8. B. Knoll *et al.*, *Nat. Neurosci.* **9**, 195 (2006).
9. S. Alberti *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6148 (2005).
10. S. Li, S. Chang, X. Qi, J. A. Richardson, E. N. Olson, *Mol. Cell. Biol.* **26**, 5797 (2006).

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PSYCHOLOGY

Birth Order and Intelligence

After nearly a century of debate, a large study shows that birth order influences intelligence, but the reasons remain to be resolved.

Frank J. Sulloway

Research on birth order and intellectual performance is replete with contradictory findings and long-standing conceptual disagreements. In the wake of these ongoing controversies, a new study that has profited from past debates is especially welcome. In an elegantly designed analysis of 241,310 Norwegian 18- and 19-year-olds that appears on page 1717 of this issue, Kristensen and Bjerkedal show that older siblings have higher intelligence test scores than younger siblings (*1*). In addition, these two researchers demonstrate that how study participants were raised, not how they were born, is what actually influences their IQs.

In a companion study, Bjerkedal *et al.* (*2*) show that birth-order differences in their Norwegian sample are nearly identical for a subset of adjacent siblings who were raised together (127,902 individuals) and for a between-family sample (112,799 individuals). Critics have long argued that such birth-order effects, which typically emerge in between-family studies, are spurious—phantom artifacts of uncontrolled differences in family size, socioeconomic status, parental IQ, and other background factors (*3–5*). At least in the domain of intellectual ability, the new Norwegian findings rule out this alternative explanation.

Critics might still argue that the mean IQ difference documented between a Norwegian firstborn and a secondborn is only 2.3 points. Such a modest difference, however, can have far greater consequences than most people realize. For example, if Norway's educational system had only two colleges—a more prestigious institution for students with IQs above the mean, and a less desirable institution for all other students—an eldest child would be about 13% more likely than a secondborn to be admitted to the better institution (the relative risk ratio), and the odds of a firstborn being admitted would be 1.3 times as great. In medicine, new therapeutic benefits of this magnitude often make front-page headlines. In addition, such differences in opportunities gained or lost inevitably accumulate over one's lifetime.

One puzzle highlighted by these latest findings is why certain other within-family studies have failed to show equally consistent results. Some of these previous null findings, which have all been obtained in much smaller samples, may be explained by inadequate statistical power, as Bjerkedal *et al.* themselves suggest. But most previous researchers have overlooked another intriguing reason for such inconsistent outcomes, which are generally found in studies of children rather than adults. As has been noted by Zajonc and colleagues, younger siblings tend to score higher than

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IQ-related effects	Confluence theory	Resource dilution theories	Prenatal theories	Spurious association theories
1. Birth-order differences in within-family data	✓	✓	✓	✗*
2. Functional versus biological birth-order differences**	✓	✓	✗	✗
3. Family-size effects	✓	✓	✓	✓
4. Only-child deficit	✓	✓	✗	✓
5. Twin deficit	✓	✓	✓	✗
6. Birth-order differences dissipate with large age spacings	✓	✓	✗	✗
7. Reversal of birth-order differences by age	✓	✗	✗	✗

*Such explanations predict limited (but spurious) birth-order differences in between-family data only.
**As demonstrated by Kristensen and Bjerkedal in this week's issue.

Sibling differences. The efficacy of models predicting differences in intellectual performance. Classifications are based on whether each theory offers a possible explanation for reported IQ differences, although not necessarily a correct explanation.

older siblings when tests of intellectual ability are conducted under the age of about 12 (*6*, *7*). In more than 50 previous samples, there is a significant tendency for IQ disparities by birth order to reverse direction as children get older.

Zajonc's own confluence model of intellectual ability provides a possible explanation for this curious age-related reversal in birth-order effects. According to this model, the family's overall intellectual environment embodies a dynamic aspect that includes all of its members' relative contributions. For example, the intellectual environment of a firstborn at, say, age 7 is actually less favorable than the environment of a 2-year-younger sibling at the same age. This is because the younger sibling, being linguistically and cognitively less mature, degrades the firstborn's intellectual environment, whereas the older sibling enriches the secondborn's environment. To explain why older siblings eventually tend to overtake their younger siblings in intellectual performance, Zajonc's model posits a tutoring effect, which kicks in as older siblings begin to teach what they know to their younger brothers and sisters. Through the organization and expression of thoughts, teaching younger siblings is posited to benefit the tutor more than the learner, especially since lastborns have no one to tutor.

Given the latest findings from Norway, it is useful to compare the features of various competing theories about birth order and intelligence and to assess how they now stack up. These alternative explanations include family resource dilution models (of which the confluence model is a sophisticated variant), theories about prenatal influences, and "admixture" theories asserting that birth-order effects are spurious products of uncontrolled confounding influences. As shown in the table, resource dilution models and the confluence model both do well in providing possible explanations for birth-order differences, as well as for other family-related effects in intelligence (*8*). For example, both models are consistent with the fact that children without siblings, who are more likely than other children to grow up in single-parent homes and who also lack a sibling to tutor, generally exhibit lower test scores than firstborns having a younger sibling. Similarly, twins are expected to score lower than singletons, either because of gestational factors (twins compete for resources inside the womb) or because they dilute the family's intellectual environment more than do singletons. Without going into further detail about the relative merits of the various models outlined in the table, it is nevertheless noteworthy that only the confluence model addresses the apparent reversal in intellectual performance by birth order as children are growing up.

The confluence model has been criticized repeatedly over the past three decades (*4*, *5*). Although this embattled model has survived these critiques, it is not without unresolved problems. One difficulty is the absence of any direct evidence showing that tutoring by older siblings actually raises their IQs, although indirect evidence is suggestive (*9*). A plausible alternative to the supposed effects of tutoring involves competitive niche partitioning within the family. Well-designed within-family studies have consistently shown that firstborns are rated by themselves, their parents, and their siblings as being more self-disciplined, hard-working, and intelligent than their younger siblings, and also as being "the achievers" of the family (*10*–*12*). Although such perceived sibling differences might well reflect differences in family roles, or even sibling stereotypes, rather than real or permanent differences in personality or ability, such competitive role differentiations and shared beliefs may also help to explain why elder siblings, by early adulthood, have higher IQs than their younger siblings.

Thanks to the new results, we no longer need to wait for truly persuasive data to justify those theories that consider birth-order differences in intellectual performance to be a within-family phenomenon. It seems likely, however, that portions of past theories—formerly uncompromising rivals—may be required within any theory that is adequate to the task of explaining findings from large national samples. For example, parents who tend to have small families may, on average, have higher IQs than do other parents, contributing to family-size effects in both between- and within-family data (*5*). Similarly, gestational factors may no longer provide a plausible explanation for birth-order effects relating to intelligence, but they are still relevant to understanding why twins have lower IQs than singletons. The greatest challenge that now confronts birth-order researchers is to find, and to creatively mine, other large data sets like that available in Norway, so that alternative explanations can be tested against one another, allowing some of these adversarial rivals a continuing, if more restricted, role in a multifaceted explanation.

References

1. P. Kristensen, T. Bjerkedal, *Science* **316**, 1717 (2007).
2. T. Bjerkedal, P. Kristensen, G. A. Skjeret, J. I Brevik, *Intelligence*, 10.1016/j.intell.2007.01.004.
3. C. Ernst, J. Angst, *Birth Order: Its Influence on Personality* (Springer-Verlag, Berlin, 1983).
4. R. D. Retherford, W. H. Sewell, *Am. Sociol. Rev.* **56**, 141 (1991).
5. J. L. Rodgers, H. H. Cleveland, E. van den Oord, D. C. Rowe, *Am. Psychol.* **55**, 599 (2000).
6. R. B. Zajonc, H. Markus, G. B. Markus. *J. Pers. Soc. Psychol.* **37**, 1325 (1979)
7. R. Bull Zajonc, F. J. Sulloway, *Pers. Soc. Psychol. Bull.*, 10.1177/0146167207303017.
8. R. Hertwig, J. N. Davis, F. J. Sulloway. *Psychol. Bull.* **128**, 728 (2002).
9. T. E. Smith, *Soc. Psychol. Quart.* **56**, 77 (1993).
10. D. L. Paulhus, P. D. Trapnell, D. Chen, *Psychol. Sci.* **10**, 482 (1999).
11. F. J. Sulloway, in *Conceptual Challenges in Evolutionary Psychology: Innovative Research Strategies*, H. R. Holcomb III, Ed. (Kluwer, Dordrecht, Boston, 2001), pp. 39–83.
12. M. D. Healey, B. J. Ellis, *Evol. Hum. Behav.* **28**, 55 (2007).

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Current Problems in the Management of Marine Fisheries

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The public perception of fisheries is that they are in crisis and have been for some time. Numerous scientific and popular articles have pointed to the failures of fisheries management that have caused this crisis. These are widely accepted to be overcapacity in fishing fleets, a failure to take the ecosystem effects of fishing into account, and a failure to enforce unpalatable but necessary reductions in fishing effort on fishing fleets and communities. However, the claims of some analysts that there is an inevitable decline in the status of fisheries is, we believe, incorrect. There have been successes in fisheries management, and we argue that the tools for appropriate management exist. Unfortunately, they have not been implemented widely. Our analysis suggests that management authorities need to develop legally enforceable and tested harvest strategies, coupled with appropriate rights-based incentives to the fishing community, for the future of fisheries to be better than their past.

The United Nations Food and Agriculture Organization (FAO), which monitors the state of world fisheries, has estimated that since 1990 approximately one-quarter of fish stocks have been overexploited, depleted, or are recovering from depletion (17%, 7%, and 1%, respectively) (*1*), with the Northeast and Northwest Atlantic, the Mediterranean, and the Black Sea being the areas with the largest number of depleted stocks (*2*). Many authors have elaborated on these conclusions, documenting the poor state of fisheries worldwide (*3*). Nevertheless, the situation, although serious, is not catastrophic, and there are grounds for optimism. There have been successes of fisheries management, and there is an understanding of what is involved in successful fisheries management and of the requirements for its implementation. These issues are explored in this review.

The management of commercial fisheries clearly requires a good scientific understanding of the behavior of the exploited stock or stocks. The science that is used to assess commercially exploited species is still dominated by the population models developed by Beverton and Holt for single-species assessments some 50 years ago (*4*). The availability of substantial computing power has meant that sophisticated estimation methods can be used, and an appreciation of the way in which fish stocks respond to environmental variability is readily incorporated in scientific advice (*5*). Calls for a more ecosystem-orientated approach have been voiced for some while, but the paucity of data and the demands of multiparameterized multispecies models means that most ecosystem considerations in practical stock assessment tend to be ad hoc manipulations of the single-species approach (*6*).

What has developed is a realization that effective management requires an understanding of how the fishery system is performing relative to reference points. The most commonly used reference points are those relating to the size of the stock itself and the fishing mortality that will result in these stock sizes, given existing relationships between the stock, recruitment, natural mortality, and growth (Fig. 1). A typical "target reference point" is the biomass necessary to produce maximum sustainable yield (BMSY). However, such targets do not explicitly recognize threats to the stock. To address this issue, stock size "limit reference points" are usually defined or interpreted as the stock biomass below which recruitment becomes substantially reduced. Clearly, it is important to avoid situations where the stock is at or below this level. Accordingly, management should aim to have as a target a level of stock size that carries a low risk (allowing for scientific uncertainty) of the stock dropping below the limit reference point (*7*). This could mean having a target level of fishing mortality that provides stock sizes above BMSY.

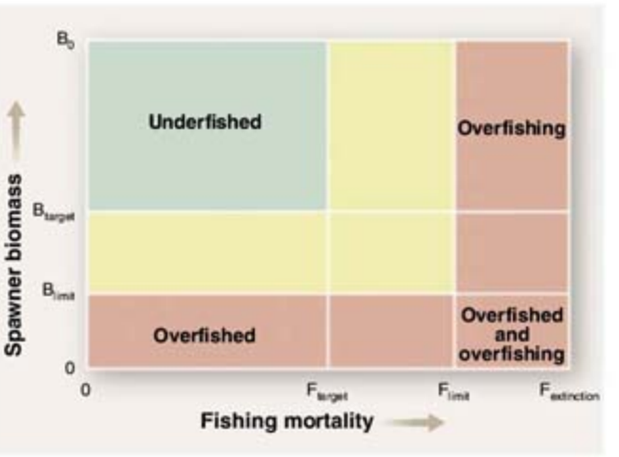

Fig. 1. Typical reference points and stock status definitions for stock biomass and fishing mortality. The limit of fishing mortality that generates biological extinction is F extinction.

Understanding Fisheries Management

Competent scientific advice based on appropriate data is far from ubiquitous in the fisheries world, and even in ideal situations, fisheries management has often been unsuccessful. The success of a management system is often defined in terms of biological, economic, social, and political objectives. Clearly, economic and social objectives will not be met while a stock is in such a depleted state that the long-term sustainability of the fishery is threatened, but equally, biological objectives are unlikely to be met without consideration being given to economic and social objectives. Hence, we argue that an understanding of the fishery management process can only come from analyzing the capacity and incentives of the two key stakeholders: the fishing community and the management authority. This is not to belittle the importance of other stakeholders, such as recreational fishers and environmental groups, who have important roles in the management of certain fisheries.

Where management is weak or nonexistent, the economic factors underlying overfishing in commercial fisheries have been generally understood since the 1950s (*8*). In short, when multiple fishers compete to catch fish from a given population, each fisher maximizes his net income by continuing to fish as long as the value of his catch exceeds the cost of catching it. An equilibrium, called the bionomic equilibrium, is reached only when fishing has reduced the fish population to a level at which catch rates are barely sufficient to cover the costs of fishing. The population is then maintained at this level through biological processes of natural growth and reproduction. Thus, if the price:cost ratio is high, the bionomic equilibrium will result in a low stock of fish, and hence a low annual catch level; two characteristic features of overfishing. In addition, the so-called economic rents (total revenue minus total costs) from the fishery will equilibrate at zero, resulting in minimal overall economic efficiency.

Many management authorities seek to meet their objectives by setting output controls in terms of a total allowable catch (TAC) for the year and closing the fishery when the year's cumulative catch has reached the TAC. Restrictions on fishing gear, fishing season, and fishing areas, as a supplement to the TAC, may also be imposed. If the TAC is correctly specified and enforced, this

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method should maintain a stock level well above that of bionomic equilibrium. If, however, the TAC and the science behind it are not respected by fishermen and not adequately enforced by authorities, widespread illegal fishing can occur. A recent example is in the eastern Baltic cod fishery where illegal fishing contributes to true catches being some 35 to 40% higher than reported (*9*).

Efficient enforcement can be difficult. Put simply, fishers will be deterred from breaking fishing regulations if their expected loss from detection and successful prosecution exceed their expected gain. In many fisheries, the probability of detection of illegal activity and the penalties are not sufficiently high to act as a disincentive (*6*).

Strong management can ensure that biological targets are met, but it is essential that regulations are enforceable, and this has often proved to be difficult. Less-than-perfect enforcement can lead to illegal fishing, poor scientific data, and a failure to meet biological targets. Input measures, such as limiting the number of vessels or restricting available season length, are usually more easily enforceable than output measures such as TAC (*10*). However, control via input measures is vulnerable to effort creep, whereby operators increase the fishing power of their vessels through technical means. Nevertheless, monitoring of vessel performance over time and adjusting the allowable level of effort have allowed successful effort control to be implemented (*11*).

Overcapacity

Simplistically, it would seem that positive economic rents should also emerge in a TAC-regulated fishery. In reality, many TAC-regulated fisheries have experienced an unexpected increase in fishing capacity, as additional vessels enter the fishery in response to (temporarily) positive rents. Economic models (*12*, *13*) then predict a regulated bionomic equilibrium, in which economic rents (net of fixed costs) again equilibrate at, or near, zero. This situation currently exists in many of the world's regulated fisheries; overcapacity of fishing fleets is widely perceived as a major impediment to achieving economically productive fisheries (*14*). It is thus ironic that such overcapacity is usually generated by the management system itself, although it can also result from high profitability during the initial phase of a newly developing fishery.

Overcapacity is widely recognized as a major problem affecting world fisheries. With its attendant social and economic problems, overcapacity can, via the political process, lead to the erosion of management control (*15*). It is also understood to be one of the results of subsidizing fisheries, which even today is estimated to be several tens of billion U.S. dollars per year (*16*). Such subsidies directly undermine the sustainability of fisheries because they lead to a bioeconomic equilibrium with high levels of fishing and low stock size. In several fisheries, government funds have been used to buy out excess fishing capacity. For various reasons, such buyback programs have been less effective than expected. First, often only the least efficient vessels are bought up, leaving total fishing capacity largely intact. Second, the buyback program by itself does not remove the economic incentives underlying overcapacity, which tends to increase once the buybacks are completed (*12*, *17*).

Thus, the underlying cause of the dual crisis of overfishing and overcapacity, as well as other undesirable outcomes, such as habitat destruction and incidental kills of untargeted species, can be found in the economic incentives of fishers who compete for their annual catches. These incentives are not affected by management strategies that retain the competition between fishers for a common-pool resource. Perhaps the most important development in fisheries management over the past 20 years has been the recognition of this fact and the introduction of rights-based management in several regimes. Indeed, it has been argued that of the tools at the disposal of managers, more emphasis needs to be placed on incentive-based approaches that better specify community and individual harvest or territorial rights, in additional to public research, monitoring, and effective administrative oversight (*18*).

Transferable Quotas

An alternative management strategy based on individually allocated transferable annual catch quotas (ITQs, or individual transferable quotas) is now in effect in several fishing nations, including Australia, New Zealand, Iceland, Canada, and Namibia. A well-organized rights system alters the economic incentives of fishers, who no longer compete for their catches, so that highly competitive fishing no longer takes place. The guarantee to fishers of a certain proportion of the catch allows them to make rational economic choices about where and when they catch fish. An ITQ system goes further, allowing the industry to settle on a fleet capacity that optimizes individual economic yield to vessels or cooperatives, although this of course can still be distorted by inappropriate subsidies.

In addition, ITQ fishers may often be expected to favor management actions that protect and enhance fish populations, because the value of a quota share increases as stocks become more abundant. Problems that may arise, such as misreporting or high-grading of catches, have been successfully countered by the use of observers, required by the management system but paid for by the industry; observers are used extensively in the U.S. Pacific fisheries, Australia, and New Zealand. Experience with ITQ systems shows that many fishers willingly support and adhere to conservative management strategies and may also avoid fishing practices that endanger habitat or threaten other species, so long as they are guaranteed long-term rights. But this does not mean that enforcement and scientific monitoring are unnecessary in ITQ systems; both are essential unless catch levels are set at precautionary low levels. It is thus unsurprising that the two countries with perhaps the most fully developed ITQ systems, New Zealand and Iceland, have some of the highest costs of management per fishing vessel (*19*)

Several authors have pointed to instances of successful fisheries management in both the developed (*20*, *21*) and developing (*22*) world. Among their conclusions are that incentive structure, institutional capacity, and participation of stakeholders are of key importance. However, in some studies, a rights-based approach is seen as the primary mechanism to deliver this (*18*), whereas in others, severe top-down controls with very limited participation of fishing communities in the management process are advocated (*23*). We argue here that a necessary condition for successful management contains all these elements: a competent management authority able to set and enforce regulations and monitor the status of the stock, together with some form of rights-based allocation to fishing operators (either collectively or individually) to avoid the situation where overcapacity produces economic hardship and erodes management capacity.

Evidence from Fisheries Performance

Reviews of successful fishery management are of necessity specific to individual fisheries and sometimes anecdotal. However, in some large areas, a combination of strong state governance and wealth, substantial scientific activity, and different types of fishery management offer the opportunity of some comparison between different types of fishery management that goes beyond the anecdotal.

Detailed data on the status of different fisheries are published for U.S., Northeast Atlantic, Australian, and New Zealand fisheries. The approach to management taken by these authorities is varied. New Zealand has the most developed and widespread application of individual user rights (ITQs), which have been in place from 1986 and have spawned other developments such as collaborative and alternative research by stakeholders (*24*). ITQs are present also in some Australian fisheries, a very few U.S. fisheries, and some Northeast Atlantic fisheries. (The Faroes, Norway, Iceland, and United Kingdom have rights-based systems, and some fleets, notably the Dutch flatfish and Spanish Grand Sole fleets, are also managed via ITQ.)

If a broad view is taken of these management areas, the evidence for the positive benefits of ITQs in supporting sustainable resource use is mixed. Only 15% of New Zealand's stocks within the quota management system, for which the stock status is known, are substantially below the target reference level. For other administrations, the percentage of stocks that are below the limit reference level (i.e., overfished), out of the total number of stocks for which the status is currently

known, is 19% for Northeast Atlantic fisheries managed by non–European Union (EU) administrations (Iceland, Faroes, and Norway), 25% for federally managed U.S. fisheries, 30% for Northeast Atlantic fisheries managed primarily by the EU, and 40% for Australian Commonwealth fisheries (*25*, *26*) (see supporting online material). Even within the United States, there are very large regional differences: 40% of major Fish Stock Sustainability Index (FSSI) stocks managed by the New England and Mid-Atlantic Fishery Management Councils, for which the stock status is known, are overfished, and 30% are subject to overfishing. By contrast, only 13% of FSSI stocks managed by the Pacific, West Pacific, and North Pacific Management Councils are overfished, with 6% suffering from overfishing.

Only the United States has seen an improvement in performance over the past several years; in 2000, 38% of U.S. stocks for which the status was known were classified as overfished. All other areas have experienced some increases in the number of overfished stocks in the past decade, although the increases in New Zealand have been very small and are offset by recoveries in some inshore stocks. However, these statistics disguise a quite dynamic situation within each region; for example, of 74 U.S. stocks requiring rebuilding, biomass is increasing in 48% of them even if they have not yet achieved rebuilt status (*27*).

More detailed examination of the U.S. situation reveals that although ITQs are not generally applied, West Coast fisheries are managed by quota controls with fishing rights assigned to fishing companies or sectors, whereas in the northeast, fisheries are managed by a days-at-sea scheme and other effort controls (*28*). In terms of their current performance and the stock recovery required by the Magnusson-Stevens Act, West Coast management systems appear to be more effective than northeast coast systems: Only two of the 18 New England stocks that were overfished in 1995 have now recovered, compared to 4 of 9 stocks similarly categorized by the Pacific Fisheries Management Council (*27*).

Clearly, non-ITQ management systems do not always fail to maintain sustainable stocks, and management systems using ITQs are not always successful. The critical additional requirement appears to be a formally adopted management strategy with predefined rules for what to do in different circumstances. In New Zealand, in addition to an ITQ system, a formal harvest strategy embedded in the Fisheries Act (1996) means that rebuilding is statutorily required when the stock is below its target level (*29*). By contrast, the lack of a formally adopted harvest strategy in the Australian Southern and Eastern Scalefish and Shark fishery has led to an increase in the number of overfished stocks in that fishery over the past 10 years, despite operating with an ITQ system since 1992 (*30*). EU fisheries also lack a formal harvest strategy; although there is a commitment under the 2002 revision of the Common Fisheries Policy to develop multiannual management plans for all stocks, such plans are only currently defined for 17 of the 94 stocks that fall under EU management, and many of these have had to be negotiated during periods of stock collapse.

The key problems, i.e., the need to provide incentives to fishers to engage constructively in fisheries management and the need to have strong legal support for predefined harvest strategies, apply equally to management of stocks under national control and those in international waters under the control of Regional Fisheries Management Organisations. To our knowledge, none of the latter currently allocate rights to individual fishers, and only a few have defined and tested effective harvest strategies. Allocation problems continue to beset these high-seas fisheries and influence compliance, data availability, and transparency (*31*–*33*).

Ultimately, the most successful management approaches are likely to combine rights-based systems, creating incentives for fishers to operate efficiently and with long-term sustainability in mind, with a strong legal structure that requires the development of pre-agreed harvest strategies and decision rules that are triggered and adhered to as reference points are passed. As indicated earlier, an adequate control of fishing activities is also necessary (*19*).

Addressing Ecosystem-Based Management

In recent years, there have been many calls for much wider use of Marine Protected Areas to address the need for ecosystem-based management. We see these as a useful part of fishery regulation, but they are not a universal solution because unless the basic issues of capacity, regulation, and rights are solved, protected areas will simply displace the problem elsewhere. In this

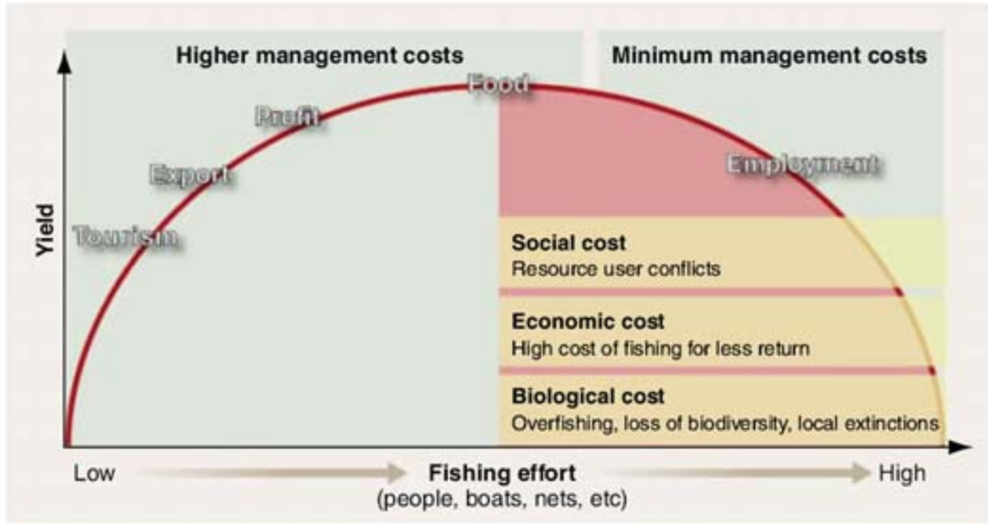

Fig. 2. The fishery management dilemma is illustrated with a simple stock production curve showing sustainable yield varying with effort. Low effort reduces biological risks and enhances economic profits at the cost of low employment and higher management costs. High effort increases employment at the cost of low economic profits and increased biological and social risks, but with low management costs (*40*).

The recovery of depleted fish stocks is a key issue and one to which most countries committed themselves in 2002 as part of the World Summit on Sustainable Development. An effective reduction in fishing effort, the participation of fishers and other stakeholders in the science and decision-making process, and the biology of the species are important factors affecting successful recovery (*34*). However, unless a harvest strategy is defined, with pre-agreed, legally binding decision rules requiring reductions of effort when stock sizes decline below limit reference points, most management authorities will still delay taking action to recover stocks. Some of this delay may arise from uncertainty in the science, but mostly it arises from an unwillingness to take decisions that will create hardship for fishers, and usually a delay will exacerbate stock decline (*35*).

we concur with recent reviews (*18*, *20*, *36*, *37*) that emphasize the primary importance of conventional measures to control fishing mortality and the secondary but essential role that marine protected areas (or other area-based management, such as local prohibitions on particular gears such as bottom trawls) have in dealing with specific issues of ecosystem conservation, such as bycatch and habitat damage.

The simple creation of rights-based incentives does not automatically deal with ecosystem problems, because fishers have little incentive to minimize bycatch or habitat damage that does not affect their target species. An interesting recent development is the creation of additional incentives for fishers through market measures, such as the creation of sustainable fisheries certification schemes and pressure from environmental nongovernmental organizations for responsible fisheries. Fishers have a major incentive to im-

prove fisheries to satisfy certification conditions, and so far most of the conditions raised in Marine Stewardship Council certifications have concerned the ecosystem effects of fishing, often related to quantifying and reducing deaths of bycatch species and damage to habitat (see supporting online material).

Even in the statistics documented for some of those states with appreciable management capacity, what is striking is for how many stocks, the status is uncertain or not determined. In the United States, the stock status of 30% of the 230 major (FSSI) stocks and stock complexes was undetermined in 2006; in Australia (48%), New Zealand (78%), and the Northeast Atlantic (61%), the numbers are even higher (*38*).

Given the problems that most authorities have in deriving reliable quantitative assessments of their stocks of major commercial importance, the large numbers of small, commercially unimportant stocks present in most areas, usually as bycatch, cannot realistically be assessed. Under a comprehensive ecosystem approach, risk assessment methodologies should be used to identify those bycatch species in need of special measures (*39*), and monitoring programs, for instance using scientific observers, need to be implemented to monitor trends in all bycatch species. The application of these approaches is in its infancy even in the most advanced management schemes; many simply respond by setting untested but hopefully precautionary effort or catch limits (*10*).

These considerations apply even more strongly to fisheries operators in developing countries. In a situation of little or no management capacity, some form of bioeconomic equilibrium is the likely result, but in such cases the management priorities may be different. Indeed, high employment with relatively modest economic rent, as long as it is compatible with the sustainability of the resource, may be a perfectly legitimate management goal (Fig. 2). In other cases, the development of Territorial Use Rights (TURFS) within local communities can lead to effective management control and rights-based operations, resulting in successful management (*20*, *21*).

Concluding Remarks

There is no doubt that there is a major problem with the world's fisheries, and, despite serious attempts to improve management and to facilitate recovery of depleted stocks, the success has been limited. The key issue that we highlight in this review is that for successful management a dual approach is required, one in which authorities provide incentives for conservation based on fishers' rights and which is supported by strong management incorporating legally enforced and tested harvest strategies.

References and Notes

1. FAO, *The State of World Fisheries and Aquaculture 2006* (FAO Fisheries Department, Rome, 2007).
2. S. M. Garcia, J. R. Grainger, *Philos. Trans. R. Soc. London B Biol. Sci.* **360**, 21 (2005).
3. D. Pauly, J. Maclean, *In a Perfect Ocean: The State of Fisheries and Ecosystems in the North Atlantic Ocean* (Island Press, Washington, DC, 2003).
4. R. J. H. Beverton, S. J. Holt, *MAFF Fish. Invest. London Ser. 2* **19**, 1 (1957).
5. T. J. Quinn II, R. B. Deriso, *Quantitative Fish Dynamics* (Oxford Univ. Press, Oxford, 1999).
6. J. R. Beddington, G. P. Kirkwood, in *Theoretical Ecology: Principles and Applications*, R. M. May, A. R. McLean, Eds. (Oxford Univ. Press, Oxford, 2007), pp. 148–157.
7. P. M. Mace, *Can. J. Fish. Aquat. Sci.* **51**, 110 (1994).
8. H. Scott Gordon, *J. Polit. Econ.* **62**, 124 (1954).
9. ICES, *Report of the Baltic Fisheries Assessment Working Group* (International Council for the Exploration of the Sea, CM 2006/ACFM:24, Copenhagen, 2006).
10. J. G. Shepherd, *Fish. Res.* **63**, 149 (2003).
11. A. J. Barton, D. J. Agnew, L. V. Purchase, in *Management of Shared Fish Stocks*, A. I. L. Payne, C. M. O'Brien, S. I. Rogers, Eds. (Blackwell, Oxford, UK, 2004), pp. 202–222.
12. C. W. Clark, *The Worldwide Crisis in Fisheries: Economic Models and Human Behavior* (Cambridge Univ. Press, Cambridge, UK, 2007).
13. C. W. Clark, *Mathematical Bioeconomics: The Optimal Management of Renewable Resources* (Wiley Interscience, New York, 1990).
14. M. H. Tupper, *Science* **295**, 1233 (2002).
15. C. Safina, A. A. Rosenberg, R. A. Myers, T. J. Quinn II, J. S. Collie, *Science* **309**, 707 (2005).
16. ICTSD, *Fisheries, International Trade and Sustainable Development: Policy Discussion Paper* (International Centre for Trade and Sustainable Development, Geneva, Switzerland, 2006).
17. C. W. Clark, G. R. Munro, U. R. Sumaila, *J. Environ. Econ. Manage.* **50**, 47 (2005).
18. R. Q. Grafton *et al.*, *Can. J. Fish. Aquat. Sci.* **63**, 699 (2006).
19. OECD, *The Cost of Managing Fisheries* (Organization for Economic Cooperation and Development, Paris, 2003).
20. R. Hilborn, *Ambio* **36**, 296 (2007).
21. R. Hilborn, J. M. Orensanz, A. M. Parma, *Philos. Trans. R. Soc. B* **360**, 47 (2005).
22. S. Cunningham, T. Bostock, Eds., *Successful Fisheries Management: Issues, Case Studies and Perspectives* (Eburon, Delft, Netherlands, 2005).
23. Pew Ocean Commission, "America's living oceans: Charting a course for sea change" (Pew Oceans Commission, Arlington, VA, 2003); available at www-ocean.tamu.edu/GOOS/GSC8/nowlin.ppt.
24. I. Del Valle, E. Hoefnagel, K. Astorkiza, I. Astorkiza, in *The Knowledge Base for Fisheries Management*, L. Motos, D. C. Wilson, Eds. (Elsevier, Amsterdam, 2006), pp. 55–83.
25. "Status of U.S. Fisheries 2006" (NOAA Fisheries, Office of Sustainable Fisheries); available at www.nmfs.noaa.gov/sfa/statusoffisheries/SOSmain.htm.
26. K. McLoughlin, *Fishery Status Reports 2005: Status of Fish Stocks Managed by the Australian Government* (Bureau of Rural Sciences, Canberra, 2007).
27. A. A. Rosenberg, J. H. Swasey, M. Bowman, *Front. Ecol. Environ.* **4**, 303 (2006).
28. See (*27*) and the New England Fishery Management Council (NEFMC) 2006 consultation document on the Magnusson-Stevens Act reauthorization (available at www.nefmc.org/nemulti/A16_scoping_final.pdf).
29. Ministry of Fisheries, "Report from the Fishery Assessment Plenary, May 2006: Stock assessments and yield estimates" (Ministry of Fisheries, Wellington, New Zealand, 2006); available at www.fish.govt.nz/en-nz/SOF/default.htm.
30. See (*26*). Note, however, that Australia is in the process of developing harvest strategies for all its stocks.
31. High Seas Task Force, "Closing the net: Stopping illegal fishing on the high seas" (Governments of Australia, Canada, Chile, Namibia, New Zealand, and the United Kingdom, World Wildlife Fund, World Conservation Union, and the Earth Institute at Columbia University, 2006).
32. D. J. Agnew, D. Aldous, M. Lodge, P. Miyake, G. Parkes, "Allocation issues for WCPFC tuna resources" (paper WCPFC3-2006-15 submitted to the 3rd regular session of the Commission for the Conservation and Management of Highly Migratory Fish Stocks in the Western and Central Pacific Fisheries Commission, Apia, Samoa, 11 to 15 December 2006); available at www.wcpfc.int/wcpfc3/pdf/WCPFC3-2006-15 [Allocation].pdf.
33. M. Mooney-Seus, A. Rosenberg, "Best practices for high seas fisheries management: Lessons learned" (Chatham House Briefing Paper, Energy, Environment and Development Programme EEDP BP 07/03); available at www.chathamhouse.org.uk.
34. C. F. Caddy, D. J. Agnew, *Rev. Fish Biol. Fish.* **14**, 43 (2004).
35. K. W. Shertzer, M. H. Prager, *ICES J. Mar. Sci.* **64**, 149 (2007).
36. C. M. Roberts, J. P. Hawkins, F. R. Gell, *Philos. Trans. R. Soc. B* **360**, 123 (2005).
37. G. Stefansson, A. A. Rosenberg, *Philos. Trans. R. Soc. B* **360**, 133 (2005).
38. The number of stocks for which the stock status is undetermined depends largely on the number of additional species and stock complexes that are recognized by management authorities. For instance, New Zealand recognizes all its possible stocks, whereas the EU has not yet recognized individual stocks for some of the important bycatch species in EU waters, such as skates and rays. In the United States, 91% of 303 non-FSSI stocks are of undetermined status (see supporting online material).
39. D. S. Kirby, B. Molony, "An ecological risk assessment for species caught in WCPO longline and purse seine fisheries" (paper EB-WP-1 submitted to the 2nd regular session of the Scientific Committee of the Western and Central Pacific Fisheries Commission, Manila, Philippines, 7 to 18 August 2006); available at www.wcpfc.int/sc2/pdf/SC2_EB_WP1.pdf.
40. C. C. Mees, R. Arthur, in *Rebuilding Fisheries in an Uncertain Environment*, Proceedings of the 13th Biennial Conference of the International Institute of Fisheries Economics and Trade, 11 to 14 July 2006, Portsmouth, UK, A. L. Shriver, Ed. (International Institute of Fisheries Economics and Trade, Corvallis, OR, 2006), paper 236.

41. We thank numerous colleagues in the fisheries science and management world whose ideas have led to this paper. We dedicate this paper to Dr. Geoff Kirkwood, who tragically died in March 2006. If he had lived, he would have coauthored this paper and improved it considerably.

Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5832/1713/DC1
SOM Text
References

10.1126/science.1137362

Explaining the Relation Between Birth Order and Intelligence

Petter Kristensen[1,2]* and Tor Bjerkedal[3]

The interest in the relation between birth order and intelligence dates back to Sir Francis Galton's *English Men of Science* (*1*). Galton found more firstborn sons in prominent positions than what he attributed to chance. This was the start of numerous studies; one of the most influential was a *Science* publication in 1973 showing a negative association between birth order and intelligence in young Dutch men (*2*). Since then, sociologists, psychologists, and demographers have proposed several explanatory models (*3*). The most influential models have emphasized explanations relating to interactions within the family and favorable conditions for intellectual stimulation for low-birth-order children.

Several researchers have claimed that the relation between birth order and intelligence is false, confounded by factors relating to family size: Families with low-intelligence children tend to be large, and the relation with birth order is an artifact when comparisons between families are made (*3*). This explanation would not produce birth order effects between siblings. Thus, the demonstration of small but notable birth order effects on intelligence quotient (IQ) in large studies examining relations within families (*4*, *5*) contradicts the idea that artifact is the full explanation.

A third model claims that the relation is explained by prenatal or gestational factors. One hypothesis suggests an effect of maternal antibody attack on the fetal brain: Maternal antibody levels tend to increase by higher birth orders in a suggested mechanism parallel to rhesus incompatibility and erythroblastosis (*6*). It has been shown that children of mothers with autoimmune disease have an increased risk of learning disabilities [for example, (*7*)], but there are no empirical data to support immunoreactivity in explaining the birth order effect.

Some children have different social and biological ranks in the family. One example is children who grow up in families with deceased elder siblings. A social interaction effect within the family would result in higher scores for a secondborn who had lost an elder sibling than for subjects ranked second both socially and biologically. On the other hand, if the birth order effect was gestational, secondborn children who are raised as the eldest would have IQ scores equal to those of other secondborn children.

We have data on birth order, vital status of elder siblings, and IQ scores among male Norwegian conscripts (*8*). This gave us an opportunity to test the family interaction and the gestational explanations. We anticipated that men who had a biological rank different from the social rank would score better than males of similar birth order who had not experienced the early loss of elder siblings if the social interaction hypothesis was right, whereas similar scores would support the gestational hypothesis. Because children from families with an adverse reproductive history had a less-advantageous distribution on a number of factors associated with low IQ (*8*), we considered it important to adjust for those factors.

Fig. 1. Relation between birth order and IQ score. Mean IQ scores for male conscripts, first-, second-, and thirdborn in Norway to mothers with single births only and first birth from 1967 through 1976, according to birth order and number of elder siblings who died in infancy (age < 1 year). Scores are adjusted for parental education level, maternal age at birth, sibship size, birth weight, and year of conscription. Error bars show 95% confidence intervals (CIs). Reference: birth order one.

IQ scores were negatively associated with both birth order and social order (table S1). Linear regression showed that these associations were stronger in adjusted models and that the effect of birth order no longer was significant ($P = 0.76$) after accounting for social order (table S1).

The adjusted IQ scores in association with all combined categories of birth order and social order are given in Fig. 1. Conscripts of first rank in social terms, no matter their biological rank, scored equal to firstborn men, albeit the confidence interval for the birth order three result was wide. Men of birth order three who grew up as the second eldest child had IQ scores close to those of secondborns with no elder sibling loss.

This study provides evidence that the relation between birth order and IQ score is dependent on the social rank in the family and not birth order as such. Furthermore, conscripts with loss of siblings are disadvantaged compared with conscripts with no such loss regarding several factors associated with intelligence. Therefore, higher scores in the former group are hardly compatible with the artifact hypothesis.

References and Notes

1. F. Galton, *English Men of Science* (MacMillan, London, 1874).
2. L. Belmont, F. A. Marolla, *Science* **182**, 1096 (1973).
3. J. L. Rodgers, H. H. Cleveland, E. van den Oord, D. C. Rowe, *Am. Psychol.* **55**, 599 (2000).
4. R. G. Record, T. McKeown, J. H. Edwards, *Ann. Hum. Genet.* **33**, 61 (1969).
5. T. Bjerkedal, P. Kristensen, G. A. Skjeret, J. I. Brevik, *Intelligence* **35**, 10.1016/j.intell.2007.01.004 (2007).
6. T. Gualtiery, R. E. Hicks, *Behav. Brain Sci.* **8**, 427 (1985).
7. G. Ross, L. Sammaritano, R. Nass, M. Lockshin, *Arch. Pediatr. Adolesc. Med.* **157**, 397 (2003).
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Genome Sequence of *Aedes aegypti*, a Major Arbovirus Vector

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We present a draft sequence of the genome of *Aedes aegypti*, the primary vector for yellow fever and dengue fever, which at ~1376 million base pairs is about 5 times the size of the genome of the malaria vector *Anopheles gambiae*. Nearly 50% of the *Ae. aegypti* genome consists of transposable elements. These contribute to a factor of ~4 to 6 increase in average gene length and in sizes of intergenic regions relative to *An. gambiae* and *Drosophila melanogaster*. Nonetheless, chromosomal synteny is generally maintained among all three insects, although conservation of orthologous gene order is higher (by a factor of ~2) between the mosquito species than between either of them and the fruit fly. An increase in genes encoding odorant binding, cytochrome P450, and cuticle domains relative to *An. gambiae* suggests that members of these protein families underpin some of the biological differences between the two mosquito species.

Mosquitoes are vectors of many important human diseases. Transmission of arboviruses is largely associated with the subfamily Culicinae, lymphatic filarial worms with both the Culicinae and the subfamily Anophelinae, and transmission of malaria-causing parasites with the Anophelinae (*1*). *Aedes aegypti* is the best-characterized species within the Culicinae (*2*), primarily because of its easy transition from field to laboratory culture, and has provided much of the existing information on mosquito biology, physiology, genetics, and vector competence (*3*, *4*). It maintains close association with human populations and is the principal vector of the etiological agents of yellow fever and dengue fever (*5*, *6*), as well as for the recent chikungunya fever epidemics in countries in the Indian Ocean area (*7*). Despite an effective vaccine, yellow fever remains a disease burden in Africa and parts of South America, with ~200,000 cases per year resulting in ~30,000 deaths (*5*). About 2.5 billion people are at risk for dengue, with ~50 million cases per year and ~500,000 cases of dengue hemorrhagic fever, the more serious manifestation of disease. The incidence of dengue, for which mosquito management is currently the only prevention option, is on the increase (*8*). Thus, there is an urgent need to improve the control of these diseases and their vector.

The availability of a draft sequence of the ~278 million base pair (Mbp) genome of *Anopheles gambiae* (*9*) has accelerated research to develop new mosquito- and malaria-control strategies. Comparisons between *An. gambiae* and *Drosophila melanogaster* (*10*) revealed genomic differences between the two insects that reflect their divergence ~250 million years ago (*11*). *Anopheles* mosquitoes radiated from the *Aedes* and *Culex* lineages ~150 million years ago (*12*), and *Ae. aegypti* and *An. gambiae* share similar characteristics such as anthropophily, but they exhibit variation in morphology and physiology, mating behavior, oviposition preferences, dispersal, and biting cycle (*1*). Both mosquito species have three pairs of chromosomes, but *Ae. aegypti* lacks heteromorphic sex chromosomes (*13*). To provide genomics platforms for research into *Ae. aegypti* and to harness the power of comparative genome analyses, we undertook a project to sequence the genome of this mosquito species.

Assembly of a draft genome sequence of *Aedes aegypti*. Whole-genome shotgun sequencing was performed on DNA purified from newly hatched larvae of an inbred substrain (LVP^{ib12}) of the Liverpool strain of *Ae. aegypti*, which is tolerant to inbreeding while maintaining relevant phenotypes (*14*). About 98% of the sequence, assembled using Arachne (*15*), is contained within 1257 scaffolds with an N50 scaffold size of ~1.5 Mbp (i.e., half of the assembly resides in scaffolds this size or longer). Assembly statistics for the 1376-Mbp genome are given in table S1. Data related to the genome project have been deposited in GenBank (project accession number AAGE00000000).

The genome size of *Ae. aegypti* as determined by sequence analysis is larger than the

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original estimate, ~813 Mbp, which was based on C_0t (DNA reassociation kinetics) analysis carried out in 1991 (*16*). An overinflated genome size could arise from assembled sequence data as a result of allelic sequence polymorphism present in a heterogeneous population of mosquitoes being sequenced. Although the estimate of 1376 Mbp may contain some such regions, we do not believe that our estimate is out of range by a large margin for the following reasons: (i) The strain that was used for the sequencing project was highly inbred (*14*); (ii) assembled sequences that are potentially "undercollapsed" are <5% of the estimated genome size (fig. S1); and (iii) flow cytometry data from six isolates of *Ae. aegypti*, including the parent of LVP^{ib12}, indicate estimated genome sizes of 1213 to 1369 Mbp (table S2).

Genetic and physical mapping data allowed assignment, but without order or orientation, of 63, 48, 39, 43, and 45 scaffolds to *Ae. aegypti* chromosome 1 and chromosome arms 2p, 2q, 3p, and 3q, respectively (*14*). These scaffolds total ~430 Mbp in length and represent ~31% of the genome (table S3). Thus, the development of high-resolution physical mapping techniques and the generation of additional random or targeted sequence data are priorities for improving the quality of the current fragmented genome assembly and size estimate. Such progress would enable unambiguous differentiation between regions of segmental duplications and residual haplotype polymorphism.

The genome of *Aedes aegypti* is riddled with transposable elements. Transposable elements (TEs) contribute substantially to the factor of ~5 size difference between the *Ae. aegypti* and *An. gambiae* genomes. About 47% of the *Ae. aegypti* genome consists of TEs (Fig. 1 and table S4; see table S4 legend for definitions of TE family, element, and copy). *Aedes aegypti* harbors all known types of TEs that have been reported in *An. gambiae* with the exception of two DNA transposons, *merlin* (*17*) and *gambol* (*18*). Simple and tandem repeats occupy ~6% of the genome, and an additional ~15% consists of repetitive sequences that remain to be classified.

Most eukaryotic TE families characterized to date (*19*) are present in *Ae. aegypti* and more than 1000 TEs have been annotated, representing a diverse collection of TEs in a single genome (table S4). Although the majority of protein-coding TEs appear to be degenerate, more than 200 elements have at least one copy with an intact open reading frame (ORF) and other features suggesting recent transposition. About 3% of the genome is composed of ~13,000 copies of the element *Juan-A* in the Jockey family of non–long terminal repeat (LTR) retrotransposons. A tRNA-related short interspersed nuclear element, *Feilai-B*, has the highest copy number, with ~50,000 copies per haploid genome. Only one highly degenerate *mariner* element is found in *Ae. aegypti*, whereas at least 20 *mariner* elements, many with intact ORFs, were found in *An. gambiae*. TEs present in *Ae. aegypti* but missing from *An. gambiae* include the *LOA* family of non-LTR retrotransposons, the *Osvaldo* element of the *Ty3/gypsy* LTR retrotransposons (*20*), and a unique family, *Penelope* (*21*). Comparison of *Ae. aegypti* and *An. gambiae* TE sequences is consistent with the interpretation of an overall lack of apparent horizontal transfer events, as a single candidate for such events was identified (*14*); one full-length copy of the *ITmD37E* DNA transposon in *Ae. aegypti* is 93% identical at the nucleotide level to a similarly classified TE in *An. gambiae*.

Miniature inverted repeat transposable elements (MITEs) and MITE-like elements of non–protein-coding TEs in *Ae. aegypti* have terminal inverted repeat sequences and target-site duplications, features characteristic of transposition of DNA transposons. Such TEs can be mobilized to transpose in trans, by transposases encoded by DNA transposons (*22*). The latter TEs occupy only 3% of the *Ae. aegypti* genome and they are less numerous than non–protein-coding DNA elements, which occupy 16% of the genome (table S4). Thus, DNA transposons may have contributed to the expansion in size and organization of the *Ae. aegypti* genome through cross-mobilization of MITEs and MITE-like TEs.

Annotation of the draft genome sequence. The fragmented nature of the assembled genome sequence, an asymmetric distribution of intron lengths within genes (figs. S2 and S3), and the frequent occurrence of TE-associated ORFs close to genes and within introns complicated the process of automated gene modeling and often led to prediction of split or chimeric gene models. Thus, we developed a multistage genome masking strategy to minimize the negative effects of TEs and other repetitive elements before gene finding (resulting in masking ~70% of the genome sequence). We also optimized gene-finding programs via iterative manual inspection of predicted gene models relative to a training set (*14*).

Two independent automated pipelines for structural annotation resulted in the prediction of 17,776 and 27,284 gene models, respectively (*14*). We made extensive use of a large collection of ~265,000 *Ae. aegypti* expressed sequence tags (ESTs) and dipteran protein and cDNA sequences in producing and then merging the two data sets into a single high-confidence gene set, which consists of 15,419 gene models (AaegL1.1). Alternative splice forms derived from these genes are predicted to generate at least 16,789 transcripts. Table 1 lists some of the genome and protein-coding characteristics of *Ae. aegypti* and those of *D. melanogaster* and *An. gambiae*.

Gene descriptions and molecular function Gene Ontology (GO) codes were assigned computationally to predicted protein sequences by means of BLASTP comparison searches with protein databases (*14*). The functional annotation pipeline included analyses of protein domains as well as secretion signal sequence and transmembrane motifs. A total of 8332 proteins were assigned a description, 9335 proteins were assigned GO terms, 2796 were assigned as "hypothetical proteins," and 5027 were denoted "conserved hypothetical proteins."

Genes encoding proteins <50 amino acid residues in length were not included in this annotation release unless they encoded known small proteins. However, these and other genes are captured in a set of 15,396 lower-confidence gene models that is available for analysis as a supplementary release (*14*). On the basis of transcriptional mapping data and limited manual examination, we anticipate that ~5 to 10% of the second-tier models or modified versions of them represent "real" genes.

TEs contribute to complex protein-coding gene structures in *Aedes aegypti*. A striking feature of protein-coding genes in *Ae. aegypti* is the factor of 4 to 6 increase in the average

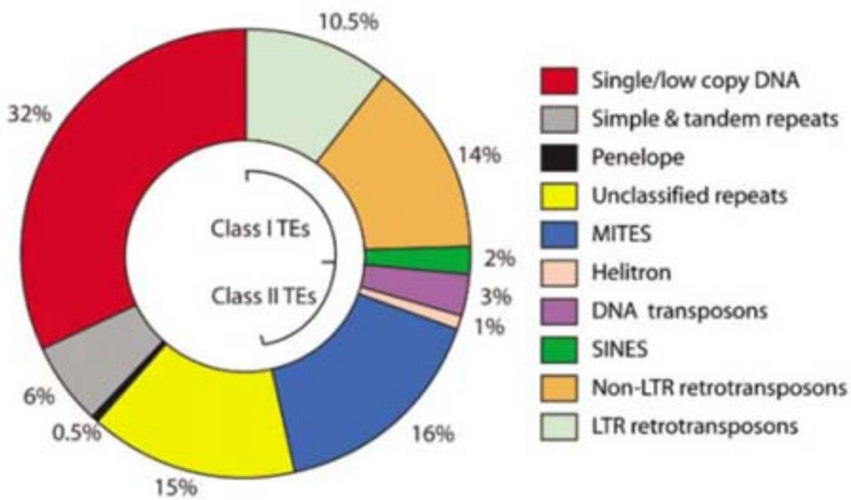

Fig. 1. Relative genomic content of annotated TEs and other sequences in *Aedes aegypti*. TEs have been deposited in TEfam, a relational database for submission, retrieval, and analysis of TEs (http://tefam.biochem.vt.edu).

length of a gene relative to *An. gambiae* and *D. melanogaster*, which is due to longer intron lengths rather than longer exons or an increased number of introns (Table 1). The increased length of introns is primarily due to infiltration by TEs; a plot of intron size before and after masking repeat sequences reveals a shift to shorter intron lengths (fig. S2). A more global perspective of the genome expansion was revealed by the difference in genomic span (factor of ~4.6) of conserved gene arrangements between *Ae. aegypti* and *An. gambiae* that occupy ~33% of each genome (table S5 and fig. S3), providing evidence that TE-mediated expansion in both genic and intergenic regions has contributed to the increased size of the *Ae. aegypti* genome. Long introns, in particular those in 5′ and 3′ untranslated regions, are likely to complicate in silico studies to define cis-acting transcription and translational regulatory elements, as they may be distant from coding sequences (fig. S4).

Transcriptional analyses. Data derived from three different transcript-profiling platforms—ESTs, massively parallel signature sequencing (MPSS), and 60-nucleotide oligomer–based microarrays—were used to experimentally confirm predicted protein-coding gene models and to gain insight into differential transcription profiles (*14*). In total, the platforms identified transcripts from 12,350 (80%) of 15,419 genes. Mapping of ~265,000 ESTs and cDNA sequences and MPSS signature sequence tags to the genome sequence as well as gene models provided evidence for transcription of 9270 and 3984 genes, respectively, whereas microarray data identified transcripts from 9143 genes (table S6). The smaller number of genes identified by MPSS (table S7) may in part be explained by the observation that only about two-thirds of the genes can be assayed by MPSS, as this approach required the presence of a Dpn II restriction enzyme site within the transcribed region. The platforms identified a common set of 2558 genes and each platform identified a unique set of genes (fig. S5), which highlights the importance of using a multi-platform approach. The data provide empirical support for ~76% of genes annotated as hypothetical (table S8), underscoring the validity of ab initio gene-finding programs in identifying novel genes.

Differences in transcript abundance between pools of RNA from nonadult developmental stages and from 4-day-old, non–blood-fed adult females were revealed by the microarray analyses, which identified 398 and 208 preadult stage and adult female enriched transcripts, respectively (table S9). Functional categorization of these transcripts differed mainly with regard to cytoskeletal, structural, and chemosensory functions (Fig. 2). Differential transcription of genes thought to be involved in chemosensory processes between these stages was conspicuous, with 17 transcripts highly enriched in mosquito developmental stages and only 3 enriched in adult females. A larger number of immune-system gene transcripts were also enriched in preadult stages (38 preadult versus 19 adult), which may reflect a broader microbial exposure of larvae and pupae in their aqueous environments. In addition, highly expressed genes encoding cuticle proteins in preadult stages (38 preadult versus 1 adult) are indicative of their function in cuticle metabolism and in a variety of other processes, including immunity, that are particularly dominant during development. The non–blood-fed status of the female mosquitoes did not enable discrimination of genes that carry out female-specific functions that mostly relate to blood processing and egg production.

***Aedes aegypti* gene families and domain composition.** Consistent with evolutionary distance estimates (*12*), there is a higher degree of similarity between the *Ae. aegypti* and *An. gambiae* proteomes than between the mosquito and *D. melanogaster* proteomes. Orthologous proteins were computed among the three genomes, with 67% of the *Ae. aegypti* proteins having an ortholog in *An. gambiae* and 58% having an ortholog in *D. melanogaster* (Fig. 3A). Analysis of three-way, single-copy orthologs revealed average amino acid identity of 74% between the mosquito proteins, in contrast with ~58% identity between mosquito and fruit fly proteins (fig. S7). About 2000 orthologs are shared only between the mosquitoes and may represent functions central to mosquito biology. Although most of these proteins are of unknown function, ~250 can be assigned a predicted function, of which 28% are involved in gustatory or olfactory systems, 12% are members of the cuticular gene family, and 8% are members of the cytochrome P450 family (*14*).

Mapping of protein domains with Interpro (*23*) revealed an expansion of zinc fingers, insect cuticle, chitin-binding peritrophin-A, cytochrome P450, odorant binding protein (OBP) A10/OS-D, and insect allergen–related domains, among others, in *Ae. aegypti* relative to *An. gambiae*, *D. melanogaster*, and the honey bee *Apis mellifera* (table S10). Some of these constitute large *Ae. aegypti* gene families, as revealed by two independent clustering methods (*14*) (table S11). Genes containing zinc finger–like domains could be of transposon or retroviral origin, and these remain to be assessed.

Species-specific differences in the number of members within a multigene family often provide clues about biological adaptation to environmental challenges. In this context, cuticle proteins have been described to play diverse roles in exoskeleton formation and wound healing and are expressed in hemocytes, a major cell type that mediates innate immunity (*24*). Cuticular proteins also are implicated in arbovirus transmission (*25*). Expansion of olfactory receptors and OBPs in *Ae. aegypti* may contribute to an elaborate olfactory system, which in turn may be linked to the expansion in detoxification capacity. The latter and insect allergen–related genes, suggested to have a digestive function, may contribute to the relative robustness of *Ae. aegypti* and also could manifest in a higher insecticide resistance. In this context, the genome and EST data have led to the development of a specific microarray to identify candidate genes among members of multigene families (cytochrome P450, glutathione *S*-transferase, and carboxylesterase) associated with metabolic resistance to insecticides (*26*). This platform will provide a means to rapidly survey mechanisms of insecticide resistance in mosquito populations and represents an important tool in managing insecticide deployment and development programs.

G protein–coupled receptors (GPCRs) that are expected to function in signal transduction cascades in *Ae. aegypti* have been manually identified (*14*). This superfamily of proteins includes 111 nonsensory class A, B, and C GPCRs, 14 atypical class D GPCRs, and 10 opsin photoreceptors (tables S12 and S13). *Aedes aegypti* possesses orthologs for >85% of the *An. gambiae* and *D. melanogaster* nonsensory GPCRs, which suggests conservation of GPCR-mediated neurological processes across the Diptera. Many *Ae. aegypti* GPCRs have sequence similarity to known drug targets (*27*) and may reveal new

Table 1. Comparative statistics of *Ae. aegypti* nuclear genome coding characteristics.

Feature	Species		
	Ae. aegypti	*An. gambiae*‡	*D. melanogaster*‡
Size (Mbp)	1,376	272.9	118
Number of chromosomes	3	3	4
Total G+C composition (%)	38.2	40.9	42.5
Number of protein-coding genes	15,419	13,111	13,718
Average gene length* (bp)	14,587	5,124	3,460
Average protein-coding gene length† (bp)	1,397	1,154	1,693
Percent genes with introns	90.1	93.6	86.2
Average number of exons/gene	4.0	3.9	4.9
Average intron length (bp)	4,685	808	1,175
Longest intron (bp)	329,294	87,786	132,737
Average length of intergenic region (bp)	56,417	17,265	6,043

*Includes introns but not untranslated regions. †Does not include introns. ‡Statistics were derived from genome updates for *An. gambiae* R-AgamP3 and *D. melanogaster* R-4.2.

opportunities for the development of novel insecticides.

Metabolic potential and membrane transporters. *Aedes aegypti* and *An. gambiae* are predicted to contain similar metabolic profiles as judged by assigning an Enzyme Commission (EC) number to both mosquito proteomes (table S14). Given the early stages of annotation, it is premature to draw conclusions from missing enzymes in predicted *Ae. aegypti* metabolic pathways. For example, assignment of EC numbers to the supplemental *Ae. aegypti* gene set (table S14) resulted in the identification of an additional 12 EC numbers (table S15) not present in AaegL1.1.

An automated pipeline (*28*) was used to predict potential membrane transporters for *Ae. aegypti* and *An. gambiae*, and their transport capacity resembles that of *D. melanogaster* (table S16). Similar to other multicellular eukaryotes, ~32% of all three insect transporters code for ion channels and probably function to maintain hemolymph homeostasis under different environmental conditions by modulating the concentrations of Na^+, K^+, and Cl^- ions. *Aedes aegypti* encodes 52 more paralogs of voltage-gated potassium ion channels, epithelial sodium channels, and ligand-gated ion channels than *An. gambiae* and 65 more such paralogs than *D. melanogaster*. These channels play important roles in the signal transduction pathway and cell communication in the central nervous system and at neuromuscular junctions. A collection of 64 putative adenosine triphosphate–binding cassette transporters was identified, including subgroups that encode multidrug efflux proteins. *Aedes aegypti* encodes 16 more members of four different types of amino acid transporters than *An. gambiae* and 13 more members than *D. melanogaster*. Mosquito larvae cannot synthesize de novo all the basic, neutral, or aromatic L–amino acids (*3*) and must rely on uptake of these essential amino acids. The richer repertoire of membrane transport systems in *Ae. aegypti* is likely to intersect with the apparent increase in odorant reception and detoxification capacity.

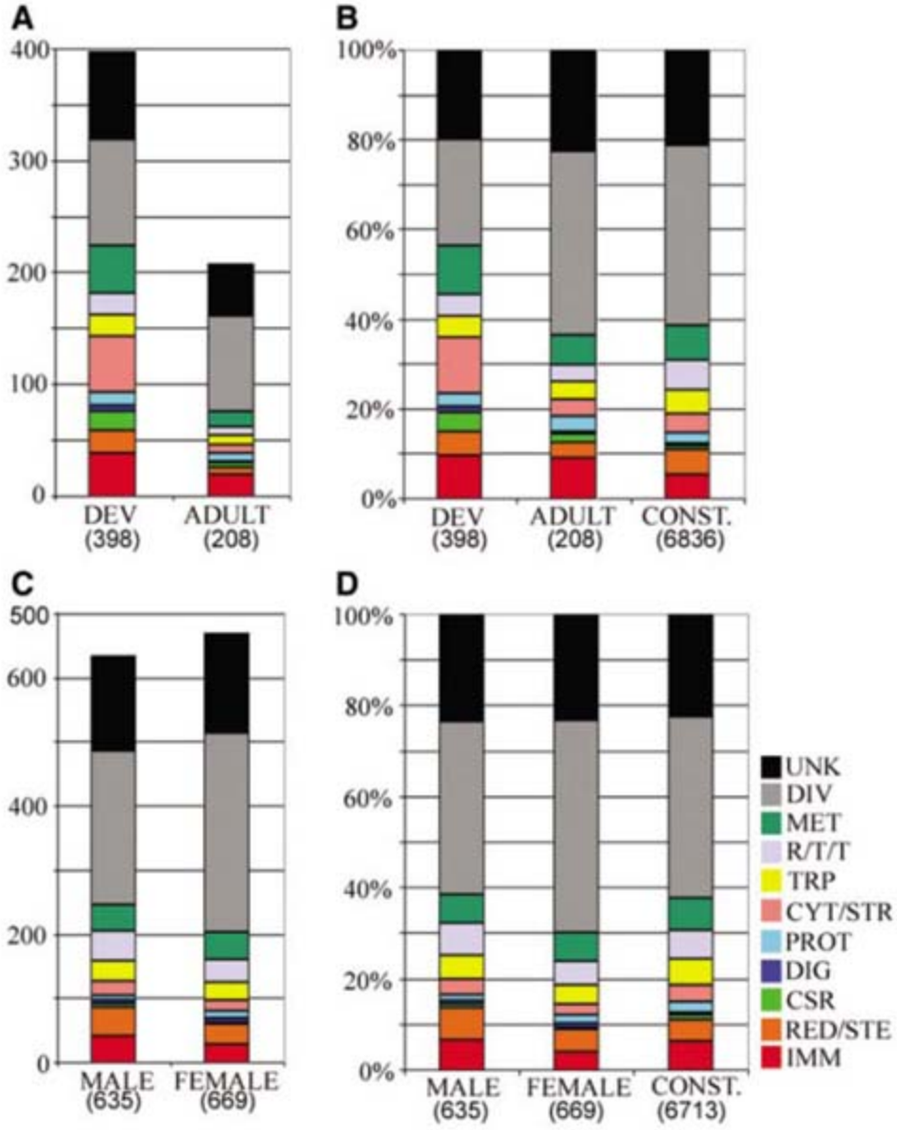

Fig. 2. Transcriptome analyses of *Aedes aegypti*. (**A**) Functional class distributions of genes that are enriched in preadult stages (DEV) and the adult female stage (ADULT) (table S9). (**B**) Proportions of functional gene classes, expressed as percentage of the total number of genes that are enriched in preadult stages (DEV), adult female stage (ADULT), and constitutively expressed genes (CONST.). (**C** and **D**) same as (A) and (B) for genes enriched in the male, in the female, and common (CONST.) for both sexes (table S18). Functional classes: IMM, immunity; RED/STE, redox and oxidoreductive stress; CSR, chemosensory reception; DIG, blood and sugar food digestive; PROT, proteolysis; CYT/STR, cytoskeletal and structural; TRP, transport; R/T/T, replication, transcription, and translation; MET, metabolism; DIV, diverse functions; UNK, unknown functions. The total number of genes in each category is indicated in parentheses.

Autosomal sex determination and sex-specific gene expression. Heteromorphic sex chromosomes are absent in *Ae. aegypti* and other culicine mosquitoes (*13*). Instead, sex is controlled by an autosomal locus wherein the male-determining allele, *M*, is dominant. The primary switch mechanism at the top of the mosquito sex determination cascade is different from that of *D. melanogaster*, where the X-chromosome/autosome ratio controls sex differentiation. However, we expect conservation of function in mosquito orthologs of *Drosophila* genes that are further downstream of the cascade (*29*). We verified the presence of a number of these in *Ae. aegypti*, including orthologs for *doublesex*, *transformer-2*, *fruitless*, *dissatisfaction*, and *intersex* (table S17).

To define gene expression differences between the sexes, we analyzed microarray transcription profiles of 4-day-old, non–blood-fed adult female and male mosquitoes (Fig. 2); 669 and 635 transcripts were enriched in females and males, respectively, and 6713 transcripts were expressed at similar levels in both sexes (table S18). An additional 373 and 534 transcripts generated exclusive hybridization signals (with signal intensity below the cutoff threshold level in one channel) in females and males, respectively, and may therefore represent sex-specific transcripts. Functional categorization of female and male enriched transcripts yielded similar results, with some exceptions; male mosquitoes expressed a larger number of immune system–related transcripts (40 in males versus 25 in females) and redox- or stress-related transcripts (45 in males versus 33 in females). By comparing the *Ae. aegypti* profiles with previously described *An. gambiae* sex-specific microarray analyses (*30*), we identified 144 orthologous genes displaying the same sex-specific transcription pattern in *An. gambiae* (table S19), whereas 74 orthologs showed an opposite profile (table S20), suggesting differences in certain sex-specific functions between the two mosquito species.

Conserved synteny with *Anopheles gambiae* and *Drosophila melanogaster*. The assignment of 238 *Ae. aegypti* scaffolds containing ~5000 genes—about one-third of the predicted gene set—to a chromosomal location on the basis of genetic and physical mapping data (*14*) allowed us to compare ortholog position and to identify

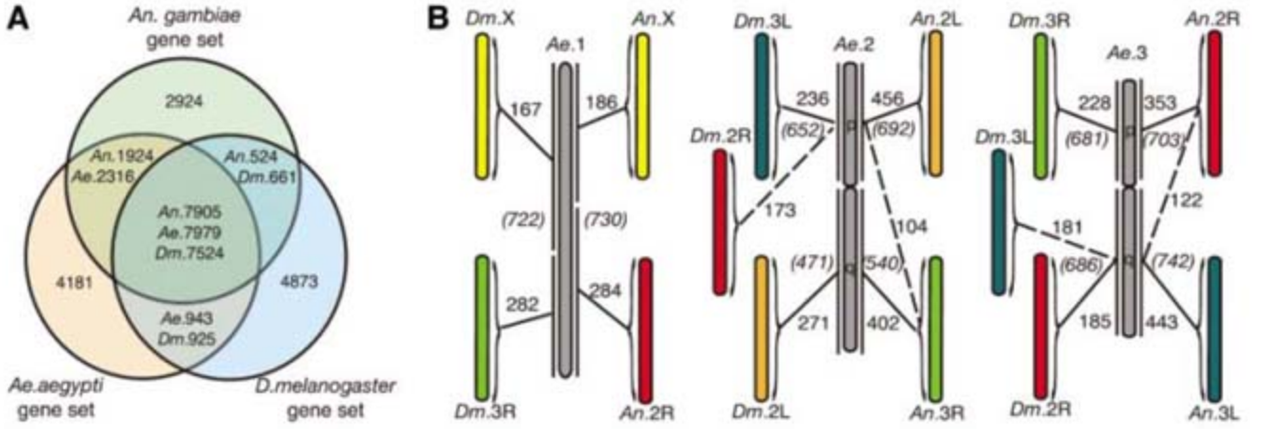

Fig. 3. Orthology and chromosomal synteny among *Ae. aegypti*, *An. gambiae*, and *D. melanogaster*. (**A**) Each circle represents a gene set for *Ae. aegypti* (*Ae*), *An. gambiae* (*An*), and *D. melanogaster* (*Dm*). Because a gene can be involved in several homologies, gene sets do not always have the same number of genes within intersections (e.g., in the *Ae-Dm* comparison, 943 *Ae* genes are similar to *Dm* and 925 *Dm* genes are similar to *Ae*). (**B**) *Aedes aegypti* chromosomes are represented in gray (not to scale). Chromosome arms are designated as "p" and "q"; chromosome 1 has no arm distinctions. Colored chromosomes represent the syntenic chromosome from *An. gambiae* or *D. melanogaster* (not to scale). Solid and dashed lines link each *Ae. aegypti* chromosome to its primary and secondary syntenic chromosome, respectively. The number of *Ae* orthologs to *An* and *Dm* chromosome arms is indicated, and the total number of orthologs on the *Ae* chromosome arm to *Ae* or *Dm* is shown in italics and parentheses.

conserved evolutionary associations between *Ae. aegypti* and *An. gambiae* or *D. melanogaster* chromosomes (tables S3 and S21). Most of the *Ae. aegypti* chromosome arms, with the exception of 2p and 3q, exhibited a distinct one-to-one correlation with *An. gambiae* and *D. melanogaster* chromosome arms with respect to the proportion of orthologous genes conserved between chromosome arm pairs (Fig. 3B). These findings confirm and extend previous results that compared a small number (~75) of *Ae. aegypti* genes with orthologs in *An. gambiae* and *D. melanogaster* (*31*).

Maps of conserved local gene arrangements (microsynteny) were computed by identifying blocks of at least two neighboring single-copy orthologs in each pair of genomes and allowing not more than two intervening genes (*14*). In line with the species divergence times, twice as many orthologs are similarly arranged between these mosquito species than between either of them and the fruit fly (table S22) (*32*); 1345 microsyntenic blocks were identified between *Ae. aegypti* and *An. gambiae*, containing 5265 out of a total of 6790 single-copy orthologs (tables S5 and S22). When *D. melanogaster* is used as an outgroup to count synteny breaks that have occurred in each mosquito lineage since their radiation, the data indicate a rate of genome shuffling in the *Ae. aegypti* lineage greater by a factor of ~2.5 than that in the *An. gambiae* lineage (*14*). However, this estimate may be inflated because of the fragmented nature of the current *Ae. aegypti* genome assembly. Thus, the highly repetitive nature of the *Ae. aegypti* genome appears to have facilitated local gene rearrangements, but it does not appear to have had a gross influence on chromosomal synteny.

Concluding remarks. The draft genome sequence of *Ae. aegypti* will stimulate efforts to elucidate interactions at the molecular level between mosquitoes and the pathogens they transmit. This already can be seen in, for example, analysis of components of the Toll immune signaling pathway (*33*) and identification of genes encoding insulin-like hormone peptides (*34*).

We expect that the sequence data will facilitate the identification of *Ae. aegypti* genes encoding recently described midgut receptors for dengue virus (*35*). Dengue vector competence is a quantitative trait, and multiple loci determine virus midgut infection and escape barriers (*36*). Unfortunately, the fragmented nature of the genome sequence and its low gene density have precluded its use in the identification of a comprehensive list of candidate genes for vector competence phenotypes or sex determination. The sequence may be used to improve the resolution of the current genetic map (*37*) and to integrate transcriptional profiling data with genetic studies (*38*), but filling gaps in the assembled sequence remains a high priority, especially when exploring genetic variations between the sequenced strain and field populations of *Ae. aegypti*.

The ongoing genome project on *Culex pipiens quinquefasciatus*, a vector for lymphatic filariasis and West Nile virus, will provide additional resources to underpin studies to systematically study common and mosquito species–specific gene function. Such analyses should improve our understanding of mosquito biology and the complex role of mosquitoes in the transmission of pathogens, and may result in the development of new approaches for vector-targeted control of disease.

References and Notes

1. B. J. Beaty, W. C. Marquardt, *Biology of Disease Vectors* (Univ. Press of Colorado, Niwot, CO, ed. 1, 1996).
2. S. R. Christophers, Aedes aegypti *(L.): The Yellow Fever Mosquito, Its Life History, Bionomics and Structure* (Cambridge Univ. Press, Cambridge, 1960).
3. A. N. Clements, *The Biology of Mosquitoes* (Chapman & Hall, London, 1992).
4. D. W. Severson, S. E. Brown, D. L. Knudson, *Annu. Rev. Entomol.* **46**, 183 (2001).
5. O. Tomori, *Crit. Rev. Clin. Lab. Sci.* **41**, 391 (2004).
6. World Health Organization, *Dengue and Dengue Haemorrhagic Fever* (World Health Organization, Geneva, 2002).
7. B. L. Ligon, *Semin. Pediatr. Infect. Dis.* **17**, 99 (2006).
8. J. S. Mackenzie, D. J. Gubler, L. R. Petersen, *Nat. Med.* **10**, S98 (2004).
9. R. A. Holt *et al.*, *Science* **298**, 129 (2002).
10. E. M. Zdobnov *et al.*, *Science* **298**, 149 (2002).
11. M. W. Gaunt, M. A. Miles, *Mol. Biol. Evol.* **19**, 748 (2002).
12. J. Krzywinski, O. G. Grushko, N. J. Besansky, *Mol. Phylogenet. Evol.* **39**, 417 (2006).
13. G. B. J. Craig, W. A. Hickey, in *Genetics of Insect Vectors of Disease*, J. W. Wright, R. Pal, Eds. (Elsevier, New York, 1967), pp. 67–131.
14. See supporting material on *Science* Online.
15. D. B. Jaffe *et al.*, *Genome Res.* **13**, 91 (2003).
16. A. M. Warren, J. M. Crampton, *Genet. Res.* **58**, 225 (1991).
17. C. Feschotte, *Mol. Biol. Evol.* **21**, 1769 (2004).
18. M. R. Coy, Z. Tu, *Insect Mol. Biol.* **14**, 537 (2005).
19. N. Craig, R. Cragie, M. Gellert, A. Lambowitz, Eds., *Mobile DNA II* (American Society for Microbiology Press, Washington, DC, 2002).
20. J. M. Tubio, H. Naveira, J. Costas, *Mol. Biol. Evol.* **22**, 29 (2005).
21. I. R. Arkhipova, K. I. Pyatkov, M. Meselson, M. B. Evgen'ev, *Nat. Genet.* **33**, 123 (2003).
22. X. Zhang, N. Jiang, C. Feschotte, S. R. Wessler, *Genetics* **166**, 971 (2004).
23. E. M. Zdobnov, R. Apweiler, *Bioinformatics* **17**, 847 (2001).
24. L. C. Bartholomay *et al.*, *Infect. Immun.* **72**, 4114 (2004).
25. H. R. Sanders *et al.*, *Insect Biochem. Mol. Biol.* **35**, 1293 (2005).
26. H. Ranson, personal communication.
27. A. Wise, K. Gearing, S. Rees, *Drug Discov. Today* **7**, 235 (2002).
28. Q. Ren, K. H. Kang, I. T. Paulsen, *Nucleic Acids Res.* **32**, D284 (2004).
29. C. Schutt, R. Nothiger, *Development* **127**, 667 (2000).
30. O. Marinotti *et al.*, *Insect Mol. Biol.* **15**, 1 (2006).
31. D. W. Severson *et al.*, *J. Hered.* **95**, 103 (2004).
32. E. M. Zdobnov, P. Bork, *Trends Genet.* **23**, 16 (2007).
33. S. W. Shin, G. Bian, A. S. Raikhel, *J. Biol. Chem.* **281**, 39388 (2006).
34. M. A. Riehle, Y. Fan, C. Cao, M. R. Brown, *Peptides* **27**, 2547 (2006).
35. R. F. Mercado-Curiel *et al.*, *BMC Microbiol.* **6**, 85 (2006).
36. C. F. Bosio, R. E. Fulton, M. L. Salasek, B. J. Beaty, W. C. Black, *Genetics* **156**, 687 (2000).
37. D. W. Severson, J. K. Meece, D. D. Lovin, G. Saha, I. Morlais, *Insect Mol. Biol.* **11**, 371 (2002).
38. R. C. Jansen, J. P. Nap, *Trends Genet.* **17**, 388 (2001).

39. The *Aedes aegypti* genome sequencing project at the microbial sequencing centers and VectorBase was funded by National Institute of Allergy and Infectious Diseases (NIAID) contracts HHSN266200309D266030071,

HHSN266200400001C, and HHSN266200400039C and was supported in part by NIAID grants UO1 AI50936 (D.W.S.), RO1 AI059492 (A.S.R., G.D.), 5 R01 AI61576-2 (G.D.), and R37 AI024716 (A.S.R.) and by Swiss National Science Foundation grant SNF 3100A0-112588/1 (E.M.Z.). We acknowledge the excellent work of the Broad Genome Sequencing Platform and the Venter Institute Joint Technology Center. We thank C. Town, N. Hall, and E. Kirkness for critical comments and the *Aedes aegypti* research community for their enthusiastic support and willing assistance in this project. On 1 October 2006 The Institute for Genomic Research merged with the J. Craig Venter Institute. The *Ae. aegypti* genome can also be accessed at VectorBase (http://aaegypti.vertorbase.org).

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Figs. S1 to S7
Tables S1 to S23
References

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Do Vibrational Excitations of CHD_3 Preferentially Promote Reactivity Toward the Chlorine Atom?

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The influence of vibrational excitation on chemical reaction dynamics is well understood in triatomic reactions, but the multiple modes in larger systems complicate efforts toward the validation of a predictive framework. Although recent experiments support selective vibrational enhancements of reactivities, such studies generally do not properly account for the differing amounts of total energy deposited by the excitation of different modes. By precise tuning of translational energies, we measured the relative efficiencies of vibration and translation in promoting the gas-phase reaction of CHD_3 with the Cl atom to form HCl and CD_3. Unexpectedly, we observed that C–H stretch excitation is no more effective than an equivalent amount of translational energy in raising the overall reaction efficiency; CD_3 bend excitation is only slightly more effective. However, vibrational excitation does have a strong impact on product state and angular distributions, with C–H stretch-excited reactants leading to predominantly forward-scattered, vibrationally excited HCl.

Several decades of experimental and theoretical molecular collision studies culminated in the formulation of Polanyi's rules of reaction dynamics (*1*). For reactions of an atom with a diatomic molecule, the rules predict the efficiency of reactant vibrational and translational energy in driving reactions over barriers; namely, vibration can be more effective than translation for a barrier located late along the reaction coordinate, and the reverse is true for reactions with early barriers. An extension of the rules to reactions of polyatomic species becomes ambiguous as a result of the higher degrees of freedom associated with multiple types of vibrational motion. Thus, one may ask: Are different vibrational modes equivalent in their capacity to promote a polyatomic reaction?

In recent years, the issue of mode-specific or bond-selective chemistry (*2*–*5*) has been the subject of several pioneering investigations, for which the reaction of the Cl atom with methane is becoming the benchmark (*6*–*19*). For example, Simpson *et al.* found that one-quantum excitation in the antisymmetric stretch (ν_3) mode of CH_4 increases the reaction rate by a factor of ~30 (*10*). On the other hand, Zhou *et al.* observed a mere threefold reactivity enhancement for one-quantum excitation of bending (ν_4) or torsional (ν_2) modes of CH_4 and CD_4 (*18*), in contrast to 200-fold and 80-fold enhancements measured earlier (*12*, *13*). Further experiments (*17*) and a quasiclassical trajectory calculation (*20*) supported the results of Zhou *et al.* Moreover, Yoon *et al.* found that excitation of the $\nu_1 + \nu_4$ symmetric stretch-bend combination mode of CH_4 enhances reactivity toward the Cl atom roughly twice as much as does the nearly isoenergetic excitation of the antisymmetric combination $\nu_3 + \nu_4$, which itself promotes a 10-fold rate enhancement over ground-state methane (*6*). In a similar study, Yoon *et al.* observed a sevenfold reactivity increase of CH_3D when the symmetric, rather than antisymmetric, C–H stretching mode was initially excited (*8*). All these experiments, however, were performed at a fixed translational or collision energy (E_c); thus, the enhanced reactivity refers to a comparison with the ground-state reaction at the same E_c. As elegant as these experiments are, it remains uncertain whether vibrational motion is more effective in driving this reaction than translation.

We report here a series of experiments aimed to resolve this uncertainty for the $Cl + CHD_3 \rightarrow HCl + CD_3$ reaction. We first studied the ground-state reaction over a wide energy range from the threshold to about 20 kcal/mol of excess energy. Experiments were then performed for the reaction with C–H stretch-excited CHD_3, again over a range of initial E_c. To refine the comparison, we also present the results for the bend- and/or torsion-excited reactants. We performed all measurements under single-collision conditions, using the rotatable, crossed molecular-beam apparatus described previously (*21*, *22*). The Cl beam was generated by a pulsed high-voltage discharge of ~4% Cl_2 seeded in a pulsed supersonic expansion of either Ne or He at 6 atm. The CHD_3 beam was also produced by pulsed supersonic expansion of either pure CHD_3 or ~20% CHD_3 seeded in H_2 (for acceleration) at 5 atm. Both beams were collimated by double skimmers and crossed in a differential-pumped scattering chamber. E_c was tuned by varying the intersection angle of the two molecular beams. A pulsed ultraviolet laser that was operated near 333 nm probed the ground-state CD_3 product via (2 + 1) resonance-enhanced multiphoton ionization, and a time-sliced velocity imaging technique mapped the recoil vector of the CD_3^+ ion (*21*). For studies with C–H stretch-excited reactants, an infrared (IR) laser was used to excite CHD_3 directly in front of the first skimmer (*19*). For reactions with bend-excited reactants, a heated pulsed valve for thermal excitation was used instead (*18*).

Figure 1 shows two typical raw images, with and without the IR-pumping laser, of the probed $CD_3(v = 0)$ products at E_c = 8.9 kcal/mol. Superimposed on the images are the scattering directions; the 0° angle refers to the initial CHD_3 beam direction in the center-of-mass frame. Thanks to the time-sliced velocity imaging approach, even the raw data can be easily interpreted by inspection. Whereas the IR-off image is dominated by a side-scattered structure, the IR-on image exhibits two distinct ringlike features reflecting the impact of C–H stretch excitation on the reaction dynamics (*23*). A sharp forward peak now appears in the inner ring, and additional broad-scattered products form the outer ring. The

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energetics of the reaction are well defined: The reaction endothermicity is 1.73 kcal/mol, and E_c is 8.9 kcal/mol. The initial ro-vibration excitation of $CHD_3(v_1 = 1, j = 2)$ adds another 8.63 kcal/mol to the total energy for the IR-on image. By conservation of energy and momentum, these ringlike features can readily be assigned as indicated. The angular distributions of product pairs $(0, 0_0)_g$ and $(0, 0_0)_s$ (the notation of which is described in the legend of Fig. 1) from the ground-state and stretch-excited reactions can be obtained directly from the IR-off and IR-on images, respectively. However, the near degeneracy (only 0.39 kcal/mol of energy difference) of the two paired channels, Cl + $CHD_3(v = 0) \rightarrow HCl(v' = 0) + CD_3(v = 0)$ and Cl + $CHD_3(v_1 = 1) \rightarrow HCl(v' = 1) + CD_3(v = 0)$, complicates the data analysis of the IR-on inner ring. Using the threshold method (*19*), we found that ~20% of CHD_3 reactants were stretch-excited. By scaling down the IR-off distribution by 0.2 and subtracting it from the IR-on data set, the genuine distribution for $(1, 0_0)_s$ was then recovered from the overlapped inner ring. The resultant pair-correlated angular distributions are presented in the lower right panel of Fig. 1, showing totally different appearances for the two pairs, which qualitatively corroborate the HCl state-resolved (not pair-correlated) results at 4.1 kcal/mol reported by Simpson *et al.* (*11*). Integrating each distribution over all angles, weighted by the sinθ term for the solid-angle factor (*21*) (where θ is the product-scattering angle), and accounting for about one-fifth of the CHD_3 reactants being pumped, we recover the respective normalized pair-correlated integral cross section (*19*).

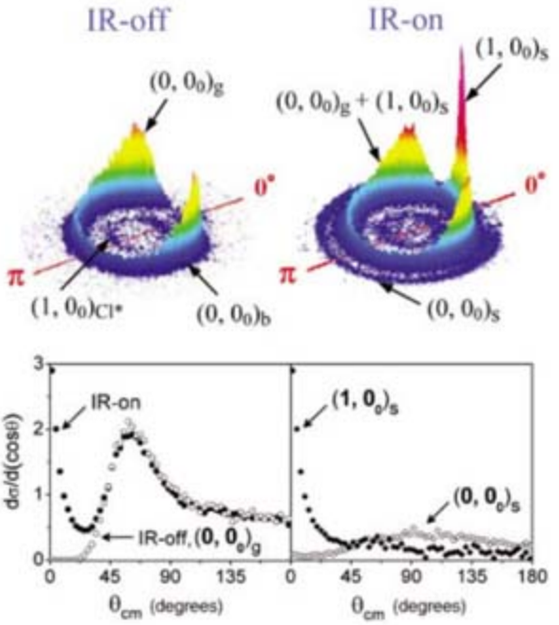

Fig. 1. (**Top**) Three-dimensional representation of the raw images, with (right panel) and without (left panel) IR-excitation, of the probed $CD_3(v = 0)$ products from the Cl + CHD_3 reaction at E_c = 8.9 kcal/mol. Based on energy conservation, the ringlike features in each image are assigned to the labeled product pairs. For clarity, the labelings $(1, 0_0)_{Cl^*}$ and $(0, 0_0)_b$ are omitted for the IR-on image. The numbers in the parentheses denote (from left to right) the quanta of vibrational excitation in HCl and the modes in CD_3 products, respectively [the inner subscript specifies the quantum of CD_3 mode and the outer subscript indicates the reactant state ("g" for ground-state CHD_3, "s" for stretch-excited CHD_3, and "b" for bend-excited CHD_3)]. (**Bottom**) The left panel shows the product angular distributions of the inner rings for the IR-on and IR-off images, and the right panel shows the deduced pair-correlated distributions from the stretch-excited reaction. $d\sigma/d(\cos\theta)$ is the differential cross section at the product-scattering angle θ_{cm} in the center-of-mass frame.

Repeating the measurements under different collision energies, normalized as stated previously (*5*, *19*), we obtain the reactive excitation function $\sigma(E_c)$, which is the dependence of the integral cross section on E_c, for the ground-state and vibrationally excited reactants (Fig. 2, A and B). Although both reactant vibrations promote reactivity, the degrees of enhancement exhibit different energy dependences, which are accentuated when plotted as ratios to the ground-state reaction (Fig. 2, C and D). Whereas the bend-excited reaction displays a nearly constant enhancement factor of ~2.5 in the post-threshold region followed by a gradual decline starting from E_c ~ 15 kcal/mol, the stretch-excited reaction efficiency drops sharply near threshold and levels off around 2 at higher E_c. Compared with previous studies, the bend result reasonably corroborates an energy-independent enhancement found for bend-excited CH_4 over an E_c range of 2.7 to 5.9 kcal/mol (*17*), and the stretch result is not entirely inconsistent with the apparently larger factors reported at single E_c for the other isotopologues (*24*).

The above-described vibrational enhancement factors are based on comparisons at the same E_c, as in all earlier studies; thus, the total available energies for the ground-state and vibrationally excited reactions are not the same. Are these factors then intrinsically mode-specific, or do they arise purely from the consideration of total deposited energy? A closer inspection, highlighted by the horizontal lines in Fig. 2, A and B, quantifies the additional translational energy needed for the ground-state reaction to proceed as efficiently as the vibrationally excited cases. This translational contribution increases from threshold to higher E_c values, suggesting an alternative and more informative view of the mode-specific enhancement factor. On an equivalent energy base, the mode-specific reactivity here refers to the differential reactivity enhancement or inhibition of the excited species relative to ground-state species that are translationally accelerated to afford an equivalent amount of total energy. Depicted in Fig. 2, E and F, are the reactivity ratios based on such a framework. Contrary to the uncalibrated ratios in Fig. 2, C and D, a very different picture emerges. At low E_c, depositing an equivalent amount of additional energy into translation turns out to be more effective in driving the reaction than exciting a

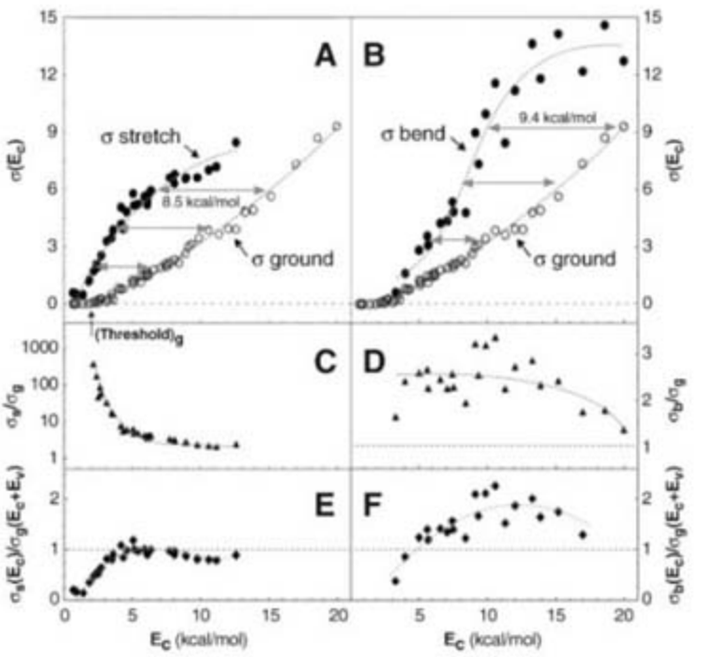

Fig. 2. Normalized reactive excitation functions for (**A**) C–H stretch-excited reactant and (**B**) CD_3 bend/torsion-excited CHD_3 as compared to the ground-state reaction. The dotted lines are visual guides. Note the characteristic step feature for a reactive resonance in the stretch-excited reaction near the energetic threshold. For the bend-excited reactant, the thermal populations (~4%) of the three low-frequency modes (ν_3, ν_5, and ν_6) are assumed (*18*); thus, the results represent the average cross sections of the three modes. The horizontal lines indicate the equivalent amounts of extra translational energy necessary to achieve reactivity observed upon vibrational excitation. Presented in (**C**) and (**D**) are the (conventional) vibrational enhancement factors for the stretch- and bend-excited reactants, respectively, where σ_s, σ_b, and σ_g are the integral cross sections for stretch-excited, bend-excited, and ground-state reactions. The preferential promotions in reactivity, based on an equivalent amount of total energy, are shown in (**E**) and (**F**), where the vibrational energies E_v are 8.63 and 3.05 kcal/mol, respectively.

stretching vibration. At higher E_c, there is no preference for either degree of freedom in dictating efficiency. This finding is unexpected in that exciting the bond to be broken in a chemical reaction would intuitively seem to be a singularly effective means of acceleration (*25*). Moreover, from the perspective of Polanyi's rules, ab initio calculations predicted a more product-like structure at the transition state (*26*–*28*); thus, a propensity for vibration over translation would be expected for this late-barrier reaction.

For the bending excitation, though the collisions at low E_c are also more sensitive to translation, at higher E_c, bending motion preferentially promotes the reactivity. The bending vibration is of lower frequency and involves nonlocalized, concerted motions of three or more atoms. The approach of the Cl atom can steer and distort the shearing motions of the CD_3 moiety. The results presented in Fig. 2F imply that a coupling of this mode to the reaction coordinate is not simply a transfer of the bending energy into translation; rather, a synergistic combination of CD_3 distortion and translation can facilitate the C–H cleavage in this direct reaction more effectively than pure translation at the same total energy. Clearly, the picture for polyatomic reactions is more complicated than the extension of Polanyi's rules would have suggested.

To shed light onto these findings, we examine the pair-correlated angular distributions $I(\theta)$ in the $I(\theta)$-θ-E_c representation (*5*, *29*, *30*). Figure 3 summarizes the results for the five product pairs probed in this study: $(0, 0_0)_g$, $(1, 0_0)_g$, $(0, 0_0)_b$, $(0, 0_0)_s$, and $(1, 0_0)_s$. A casual inspection of the $I(\theta)$-θ-E_c patterns reveals that the ground-state product pairs from all three reactions (with ground-state, bend-excited, and stretch-excited reactants, respectively) are similar, showing the direct-scattering ridge characteristic of a peripheral collision (*29*). On the other hand, the excited product pair from either the stretch-excited or the ground-state reactant displays distinct patterns with pronounced forward peaking and somewhat less backward peaking, suggestive of different reaction mechanisms. Also shown in Fig. 3 (bottom right panel) are the vibrational branching fractions of the HCl($v' = 1$) products from the stretch-excited and ground-state reactions. [Only a single product pair $(0, 0_0)_b$ was detected from the bend-excited reactant.] Both branching fractions increase abruptly near the energetic threshold and remain roughly constant with further increases in E_c. Notably, the initial stretching excitation exerts a large effect on vibrational energy disposal: The branching fraction increases 20-fold for the reaction with C–H stretch-excited CHD_3. In other words, C–H stretch excitation strongly favors a product distribution with vibrationally excited HCl.

We interpret these results using a model previously proposed for the ground-state reaction of Cl + CH_4 (*29*, *30*). Theoretical calculations on the isotopically analogous reactions suggest that the interaction with an approaching Cl atom causes rapid and strong decreases in the C–H stretching and the CD_3 umbrella-bending frequencies in the transition-state region (*8*, *26*–*28*). Based on those calculations, the model adiabatically correlates the vibrational energy curves of the reactant and product pairs by assuming that the vibrational modes preserve their character along the reaction path, as depicted in Fig. 4. Theory also predicts that these two modes not only strongly couple to the reaction coordinate through the curvature passage near the transition-state region but also couple to each other via Coriolis interactions (shaded areas in Fig. 4), fostering nonadiabatic transitions. As evidenced from the exceedingly small branching fraction for $(1, 0_0)_g$ indicated in Fig. 3, the reaction with ground-state CHD_3 is therefore largely vibrationally adiabatic, presumably because of the inefficiency of translation-to-vibration energy transfer in the entrance valley. Thus, most of the reactive flux proceeds along the ground-state potential curve, producing the $(0, 0_0)_g$ pair (*31*).

The reaction with bend-excited CHD_3 produces exclusively the ground-state $(0, 0_0)_b$ product pair, in sharp contrast to theoretical predictions of the predominant formation of umbrella bend-excited methyl radicals (*26*–*28*). However, the observed $I(\theta)$-θ-E_c pattern is very similar to

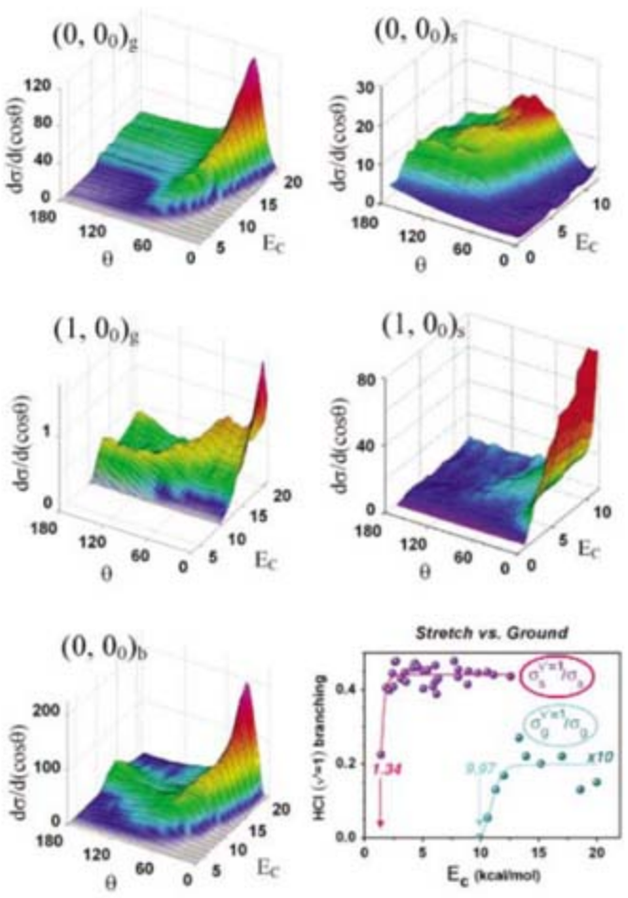

Fig. 3. Evolution of the state-correlated angular distributions as a function of collision energies, where E_c is in kcal/mol and θ in degrees. Note the difference in energy ranges and the large disparities in the vertical scales, which have been normalized to one another and to the excitation functions (Fig. 2). The energy evolutions of the angular distribution display distinct patterns: in particular, the ridge structures of the ground-state product pairs and the sharp forward-backward peaking for the HCl($v' = 1$) pairs. Also shown are the vibrational branching fractions of the coincidently formed HCl($v' = 1$) products from the stretched-excited and ground-state reactions (see the bottom right panel). The magnitude of the former reaction is nearly 20 times as large as that of the latter reaction. The vertical arrows mark the respective energetic thresholds.

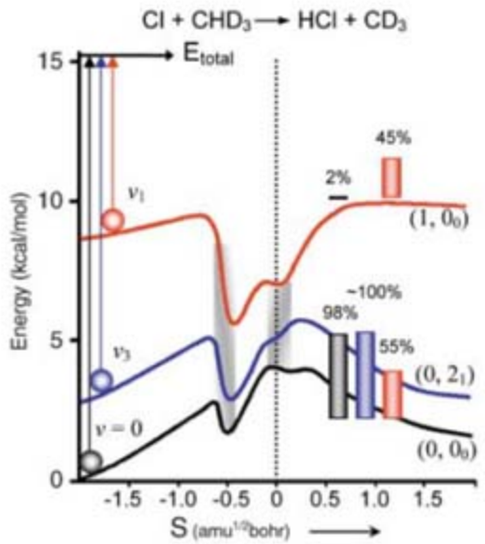

Fig. 4. Schematic representation of vibrationally adiabatic potential energy curves along the reaction coordinate *S*. The curves are depicted in keeping with the theoretically predicted vibrational frequencies with approximate isotope corrections. For clarity, only those relevant to this study are shown. Note the shifting and lowering of the barriers for the reaction with stretch-excited reactants, which might partially account for the higher cross sections at low E_c shown in Fig. 2A. The shaded areas denote the strong curvature and Coriolis couplings region, where vibrationally nonadiabatic transitions occur. Also illustrated are reactions at the same initial (total) energy from three different reactant states and the typical branching ratios of the resultant product pairs (from Fig. 3). amu, atomic mass unit.

that for the $(0, 0_0)_g$ pair, in accord with the theoretical prediction of a strong curvature coupling of this mode to the reaction coordinate (*26*–*28*). To reconcile these seemingly conflicting predictions, the experimental results suggest that the low-frequency bending vibrations of CHD_3, despite its adiabatic correlation to the $(0, 2_1)$ product pair, do not preserve their characters onto the analogous motions of the CD_3 products but rather behave as transitional modes in this reaction by promptly funneling the vibrational energy into the rotational and translational motions of the departing products.

In the stretch-excited reaction, the correlated angular distributions of the two product pairs exhibit very different patterns but bear strong resemblance to the distributions of corresponding product pairs from the ground-state reaction. By pattern comparison, we assert that the $(1, 0_0)_s$ pair is produced adiabatically with the salient forward peak resulting from a resonance state temporarily trapped by the dynamic well of the stretch-excited (red) curve in Fig. 4 (*4*, *30*, *32*). In that regard, the observation of a step-like feature in the reactive excitation function near threshold (Fig. 2A) is particularly noteworthy, because a similar feature has been observed experimentally in F + HD and confirmed theoretically as an unambiguous fingerprint for reactive resonance (*32*–*34*); this small step also echoes our recent contention for a resonance in the analogous Cl + CH_4 reaction (*30*).

In contrast, the pattern of the $(0, 0_0)_s$ pair suggests the presence of nonadiabatic pathways induced by the curvature coupling of stretching motions to the reaction coordinate (*26*–*28*). Hence, a bifurcation of reaction paths for stretch-excited reactants must occur, presumably near the entrance valley of the transition state. The vibrational branching fraction for the nonadiabatic pathway is quite substantial, $\sigma_s^0/(\sigma_s^0 + \sigma_s^1) \sim 0.55$ from Fig. 3 (where σ_s^0 and σ_s^1 are the integral cross sections for the $(0,0_0)_s$ and $(1,0_0)_s$ product pairs from the stretch-excited reaction, respectively), indicating a facile process. Theory also predicts a strong Coriolis coupling between the stretching and bending modes (*20*, *27*); this facile nonadiabatic transition could then be mediated and facilitated by the transitional nature of the bending motions of the CD_3 moiety during the course of the reaction.

References and Notes

1. J. C. Polanyi, *Acc. Chem. Res.* **5**, 161 (1972).
2. R. N. Zare, *Science* **279**, 1875 (1998).
3. F. F. Crim, *Acc. Chem. Res.* **32**, 877 (1999).
4. K. Liu, *J. Chem. Phys.* **125**, 132307 (2006).
5. K. Liu, *Phys. Chem. Chem. Phys.* **9**, 17 (2007).
6. S. Yoon, S. Henton, A. N. Zivkovic, F. F. Crim, *J. Chem. Phys.* **116**, 10744 (2002).
7. S. Yoon, R. J. Holiday, F. F. Crim, *J. Chem. Phys.* **119**, 4755 (2003).
8. S. Yoon, R. J. Holiday, E. L. Sibert III, F. F. Crim, *J. Chem. Phys.* **119**, 9568 (2003).
9. R. J. Holiday, C. H. Kwon, C. J. Annesley, F. F. Crim, *J. Chem. Phys.* **125**, 133101 (2006).
10. W. R. Simpson, T. P. Rakitzis, S. A. Kandel, T. Lev-On, R. N. Zare, *J. Phys. Chem.* **100**, 7938 (1996).
11. W. R. Simpson, T. P. Rakitzis, S. A. Kandel, A. J. Orr-Ewing, R. N. Zare, *J. Chem. Phys.* **103**, 7313 (1995).
12. S. A. Kandel, R. N. Zare, *J. Chem. Phys.* **109**, 9719 (1998).
13. Z. H. Kim, A. J. Alexander, H. A. Bechtel, R. N. Zare, *J. Chem. Phys.* **115**, 179 (2001).
14. Z. H. Kim, H. A. Bechtel, R. N. Zare, *J. Am. Chem. Soc.* **123**, 12714 (2001).
15. H. A. Bechtel, Z. H. Kim, J. P. Camden, R. N. Zare, *J. Chem. Phys.* **120**, 791 (2004).
16. H. A. Bechtel, J. P. Camden, D. J. A. Brown, R. N. Zare, *J. Chem. Phys.* **120**, 5096 (2004).
17. H. A. Bechtel *et al.*, *Angew. Chem. Int. Ed.* **44**, 2382 (2005).
18. J. Zhou, J. J. Lin, B. Zhang, K. Liu, *J. Phys. Chem. A* **108**, 7832 (2004).
19. S. S. Yan, Y.-T. Wu, K. Liu, *Phys. Chem. Chem. Phys.* **9**, 250 (2007).
20. J. Sanson, J. C. Corchado, C. Rangel, J. Espinosa-Garcia, *J. Phys. Chem. A* **110**, 9568 (2006).
21. J. J. Lin, J. Zhou, W. Shiu, K. Liu, *Rev. Sci. Instrum.* **74**, 2495 (2003).
22. J. J. Lin, J. Zhou, W. Shiu, K. Liu, *Science* **300**, 966 (2003).
23. The innermost ring labeled as $(1, 0_0)_{Cl^*}$ arises from ground-state CHD_3 reacting with spin-orbit–excited $Cl(^2P_{1/2})$, the discussion of which is beyond the scope of this report.
24. The rapid decline near threshold in Fig. 2C has an energetic origin and could account for the comparatively larger enhancement factors reported in previous studies of isotopically analogous reactions. In a typical "photoloc" (photoinitiated bimolecular reactions with the use of the law of cosines) experiment (*6*–*16*), the reaction of the Cl atom with methane has an average E_c of about 3.5 kcal/mol with a comparable energy spread. Averaging the reactivity ratio shown in Fig. 2C over this energy spread will then yield an enhancement factor that is substantially higher than its actual ratio. Similarly, the HCl($v' = 1$) branching fraction from the photoloc experiment will be somewhat smaller because of the threshold effects (Fig. 3).
25. One issue may cloud the present comparison: Only $CD_3(v = 0)$ products were probed in this work. Although these results remain a reasonable approximation to total reactivity for the ground-state reaction, the same may not hold for the stretch-excited one. A recent, preliminary investigation of the latter reaction at $E_c = 8.1$ kcal/mol indicated that the two pairs $(0, 0_0)_s$ and $(1, 0_0)_s$ probed in this work account for about two-thirds of the total product distribution, with the remainder distributed among the umbrella-excited CD_3 products. Taking this factor into account, the stretching enhancement factor at 8.1 kcal/mol shown in Fig. 2E will then rise from ~0.95 to 1.4, which is still a rather modest preferential enhancement and almost equivalent to the bending enhancement factor.
26. W. T. Duncan, T. N. Truong, *J. Chem. Phys.* **103**, 9642 (1995).
27. J. C. Corchado, D. G. Truhlar, J. Espinosa-Garcia, *J. Chem. Phys.* **112**, 9375 (2000).
28. J. F. Castillo, F. J. Aoiz, L. Banares, *J. Chem. Phys.* **125**, 124316 (2006).
29. J. J. Zhou, B. Zhang, J. J. Lin, K. Liu, *Mol. Phys.* **103**, 1757 (2005).
30. B. Zhang, K. Liu, *J. Chem. Phys.* **122**, 101102 (2005).
31. A tiny fraction, ~2% (Fig. 3), does reach the excited stretching curve and gets temporarily trapped in the dynamic well, forming a transient complex that leads to the $(1, 0_0)_g$ product pair. Its $I(\theta)$-θ-E_c pattern displays both a direct-scattering ridge and sharp forward and backward peaking, characteristic features for a short-lived complex reaction mechanism (*4*, *30*). Thus, the formation of the $(1, 0_0)_g$ pair involves contributions from both pathways.
32. S.-H. Lee, F. Dong, K. Liu, *J. Chem. Phys.* **125**, 133106 (2006).
33. R. T. Skodje *et al.*, *J. Chem. Phys.* **112**, 4536 (2000).
34. R. T. Skodje *et al.*, *Phys. Rev. Lett.* **85**, 1206 (2000).

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The Structure of Ferrihydrite, a Nanocrystalline Material

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Despite the ubiquity of ferrihydrite in natural sediments and its importance as an industrial sorbent, the nanocrystallinity of this iron oxyhydroxide has hampered accurate structure determination by traditional methods that rely on long-range order. We uncovered the atomic arrangement by real-space modeling of the pair distribution function (PDF) derived from direct Fourier transformation of the total x-ray scattering. The PDF for ferrihydrite synthesized with the use of different routes is consistent with a single phase (hexagonal space group $P6_3mc$; a = ~5.95 angstroms, c = ~9.06 angstroms). In its ideal form, this structure contains 20% tetrahedrally and 80% octahedrally coordinated iron and has a basic structural motif closely related to the Baker-Figgis δ-Keggin cluster. Real-space fitting indicates structural relaxation with decreasing particle size and also suggests that second-order effects such as internal strain, stacking faults, and particle shape contribute to the PDFs.

Ferrihydrite is ubiquitous in many near-surface environments (*1*, *2*) and is routinely used in industrial applications such as direct coal liquefaction and metallurgical processing (*3*, *4*). It occurs in pristine soils and sediments as the precursor to hematite and often in areas contaminated by acid mine drainage (*5*). Because of its extremely high surface area and reactivity, ferrihydrite plays a substantial role in the sequestration of contaminants from groundwater and streams through adsorption and coprecipitation. As such, it is also manufactured for use as a scavenger of heavy metals and metalloids during the treatment of wastewaters and in remedial activities. It is also suspected to form the inorganic core of ferritin, an iron storage protein that plays a key role in controlling the levels of iron in plants, animals, and microbes (*6*).

Even with the considerable attention given to the chemical and physical properties of ferrihydrite in previous research, there is no consensus on the crystal structure of this mineral. The primary impediment to the development of a definitive structural determination is the size of individual ferrihydrite crystallites, which are typically <10 nm (nanocrystalline). With regard to structure, most of the disagreement centers on the possible presence of multiple structural phases and the local environment of iron (*7*–*10*) and has implications for understanding its reactivity, magnetic properties, and overall chemical composition. No single formula is widely accepted for ferrihydrite; this is attributed to variable water content and a lack of a known crystal structure (*11*). Ferrihydrite is commonly designated as "two-line" or "six-line" on the basis of the number of poorly defined, broadened maxima observed in x-ray diffraction (XRD) patterns. Determining a starting structural model for this phase is particularly challenging because ferrihydrite has no known well-crystalline counterpart that can be synthesized in the laboratory or found in nature. We have recently shown that the short- and intermediate-range ordering in synthetic nanocrystalline ferrihydrite is consistent for scattering domain sizes ranging from 2 to 6 nm, and therefore the structure of ferrihydrite appears to be single phase (*12*). This result contradicts some previous studies (*7*–*10*) and is fundamentally different from the current multiphase structure model that is increasingly cited for this phase.

Synthetic ferrihydrite with three distinct average coherent scattering domain sizes of approximately 2 (Fhyd2), 3 (Fhyd3), and 6 nm (Fhyd6) were synthesized and evaluated as part of this study [see supporting online material (SOM) text]. We used the PDF method to perform a structural analysis, which involved a comparison between PDFs generated from the experimental scattering data and those calculated from structural models [see (*13*) for a review]. A highly constrained "Rietveld-like" refinement can be performed on a properly normalized PDF, $G(r)$, in which the unit cell dimensions, atomic positions, displacement, occupancies, and other model-dependent parameters are varied to improve the fit between the observed and calculated PDF (*14*). The starting model best describing the distribution of interatomic distances in ferrihydrite is isostructural to the mineral akdalaite ($Al_{10}O_{14}(OH)_2$) (SOM text) (*15*) and was identified by using the few positions of the most identifiable Bragg features in the XRD patterns. On the basis of the real-space fitting results, we contend that the structure of ferrihydrite with domain sizes ranging from 2 to 6 nm can be adequately described by a single-phase model with the hexagonal space group $P6_3mc$ and a unit cell with average dimensions of a = ~5.95 Å and c = ~9.06 Å (Fig. 1). On the basis of this structure in its ideal form, the chemical formula for ferrihydrite is $Fe_{10}O_{14}(OH)_2$. Thermal analysis strongly suggests the presence and particle-size dependence of additional surface-bound water (*12*). Although the structure of ferrihydrite can be satisfactorily described by a periodic model (Fig. 2), residuals in the fitting results suggest that second-order effects such as disorder, surface relaxation, internal strain, defects (e.g., stacking faults), particle shape (*16*), and/or interparticle correlations may also contribute to the experimental PDFs. Misfits in the region between 2.5 and ~6 Å (Fig. 2) are reminiscent of those found in a similar study of γ-Al_2O_3, in which differences were attributed to nanometer-sized domains (~1 nm) reflecting stacking faults in the matrix (*17*).

The basic structural motif of the model, which is closely related to the Baker-Figgis δ-Keggin cluster (*18*), consists of 13 iron atoms and 40 oxygens (Fig. 3). The central tetrahedrally coordinated Fe is connected by μ_4-oxo bridges to 12 peripheral octahedrally coordinated Fe atoms arranged in edge-sharing groups of three. The 2- to 6-nm ferrihydrite nanoparticles can then be described as a three-dimensional packing of these clusters with adjacent clusters connected by a common pair of edge-shared octahedra, forming μ_4-oxo bridges from the three μ_2-OH groups cis to each of the μ_4-oxo centers in the bare cluster. This arrangement creates a cubane-like moiety corresponding to four edge-shared Fe octahedra (Fig. 1). Fitting results indicate that some param-

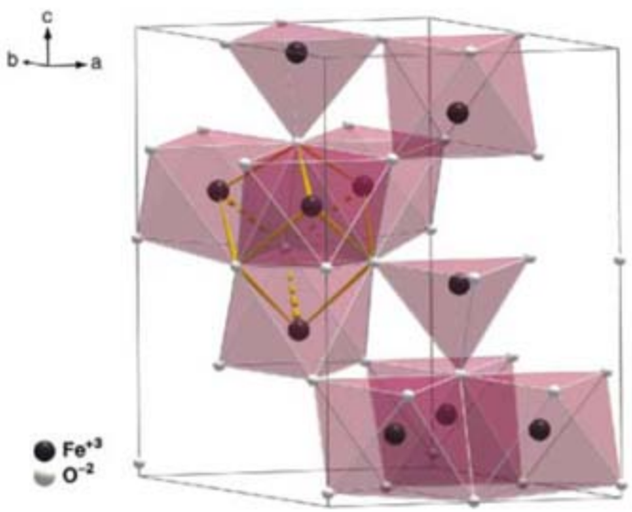

Fig. 1. Polyhedral representation of the hexagonal unit cell for ferrihydrite. The bonded atoms (yellow) define a cubane-like moiety that connects the basic structural motif of the model.

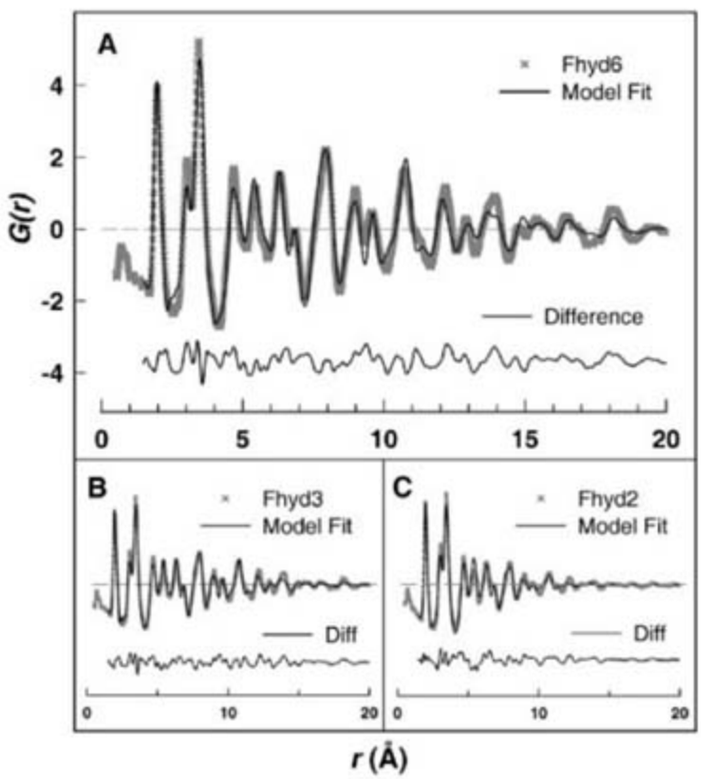

Fig. 2. The PDF, also known as $G(r)$, for Fhyd6 (**A**), Fhyd3 (**B**), and Fhyd2 (**C**) plotted to 20 Å (gray x's, which form a gray line when close together) with the refined fit of the model overlaid (solid black) for each. Difference plots are shown immediately below.

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eters such as unit cell dimensions and occupancies change systematically as the average domain size decreases from 6 to 2 nm for Fhyd6 and Fhyd2, respectively. Additionally, the degree of distortion of the Fe1 polyhedra varies as indicated in part by the change in the refined *z* parameter for the O1 site, but despite these differences, the cluster-like structural motif remains. Such changes could reflect the occurrence of stacking defects or internal strain in the structure but are not fully understood.

This structure in its ideal form consists of 20% FeO_4 and 80% FeO_6 polyhedra. However, the Fe2 and Fe3 sites show a decrease in occupancy with decreasing particle size, whereas no particle size–dependent changes are observed for the fully occupied Fe1 site. This trend might reflect an overall increase in disorder resulting from the change in ratio of Fe atoms near the surface versus interior with decreasing particle size (*19*). These surface regions of ferrihydrite are anticipated to be predominantly octahedrally coordinated given the overall topology of the structure presented and the unlikely existence of coordinatively unsaturated Fe at the hydrated surfaces. The refined parameters for each sample are available in the SOM text.

The presence of tetrahedrally coordinated iron in ferrihydrite has been the subject of considerable debate. Previous estimates based on a variety of techniques have ranged from 0 to ~40%, approximately the same amount found in maghemite (γ-Fe_2O_3), and even up to 100% (*20*–*22*). In a recent study using electron energy loss spectroscopy (EELS) to evaluate the effects of electron beam damage to ferrihydrite, Pan *et al.* (*23*) observed the reduction of Fe^{3+} to Fe^{2+} and a migration of Fe from octahedral to tetrahedral sites with increasing electron dose. These results highlight how investigations carried out under the high vacuum of the transmission electron microscope may cause substantial, and perhaps undetected, changes to occur in a sample. Such changes must now be considered when evaluating the most current and increasingly cited multiphase model for ferrihydrite that was purportedly confirmed using electron nanodiffraction (*7*, *8*). Pan *et al.* estimated, by linear extrapolation to very low electron dose (1 electron nm^{-2}), that tetrahedrally coordinated Fe^{3+} could be absent from the pristine structure of ferrihydrite and may appear only as a result of electron beam damage. However, this is a minimum estimate. Peak fitting of the energy-loss spectrum recorded at the lowest measured electron dose (3×10^4 electron nm^{-2}) indicates that, at this dose, as much as 25 ± 15% (where 15% is the experimental error) of the total iron in the mineral is tetrahedrally coordinated Fe^{3+} (*24*). Mössbauer studies performed at temperatures as low as 4.2 K by our group and others (*25*) show spectra completely split magnetically and appearing as sextets. Although this behavior does not rule out the existence of discretely different iron sites, the existence of 4-coordinated iron remains inconclusive (*26*). Future Mössbauer studies at subliquid He temperatures may prove useful for further resolving this debate, as was demonstrated in a study of a compound containing a structurally related Fe_{13} cluster ($(C_5H_6N^+)_5[Fe_{13}F_{24}(OCH_3)_{12}O_4]\cdot CH_3OH{\cdot}4H_2O$) (*27*).

Whether a 2-nm (i.e., ~30 unit cells) or even a 6-nm platelike particle can be described by a periodic model is debatable. Perhaps a particle with maximum dimensions of only several nanometers and possessing substantial disorder might better be described by a single large ensemble of atoms. We found that the satisfactory fit obtained by the single-phase defect-free unit cell for ferrihydrite in this study does not support this view. Synthetic ferrihydrite samples with average coherent scattering domain sizes ranging from 2 to 6 nm can be described by a single-phase periodic structure that does not require multiphase, size-dependent models (fig. S1 and SOM text). The proposed structure model for ferrihydrite does not address the positions for H sites. However, we anticipate that, with the iron and oxygen framework established, neutron total scattering studies on deuterated ferrihydrite will provide a complete model.

Quantitatively evaluating the structures of materials that exhibit coherent periodicity only on length scales on the order of <10 nm and that are sensitive to changes under vacuum represents a considerable challenge for conventional diffraction methods and electron imaging techniques. Although ab initio structure determination of nanocrystalline materials is in its infancy (*28*), domain-limited PDFs from total scattering data to very high reciprocal space values (i.e., ≥25 $Å^{-1}$) provide a map of interatomic distances, which can be fitted with competing structure models (*29*). Such models may appear to be quite similar in terms of short-range ordering (i.e., distances of 1 to 5 Å) but are distinguishable over intermediate-range length scales (i.e., 5 to 20 Å) because of differences in overall topology. Although it is not possible to demonstrate the uniqueness of a successful model, a solution consistent with the experimental PDF over 20 Å, or more, allows for strong confidence in the result. Traditional structure solution in well-ordered periodic materials relies on the interpretation of sharp Bragg reflections to derive an initial unit cell. This conventional approach is generally not feasible for nanocrystalline materials like ferrihydrite because of the lack of discernable Bragg reflections in diffraction patterns dominated by broad diffuse scattering. This diffuse component results not only from extremely small coherent scattering domain sizes but also from surface relaxation, strain, and complex disorder that often distinguish the structures of nanocrystalline materials from their bulk counterparts (*30*). Thus, the interpretation of the broad diffuse diffraction patterns of nanometer-sized crystals through observation of structural details in real space by means of the PDF method currently provides the best means of discrimination between existing, and potentially closely related, models.

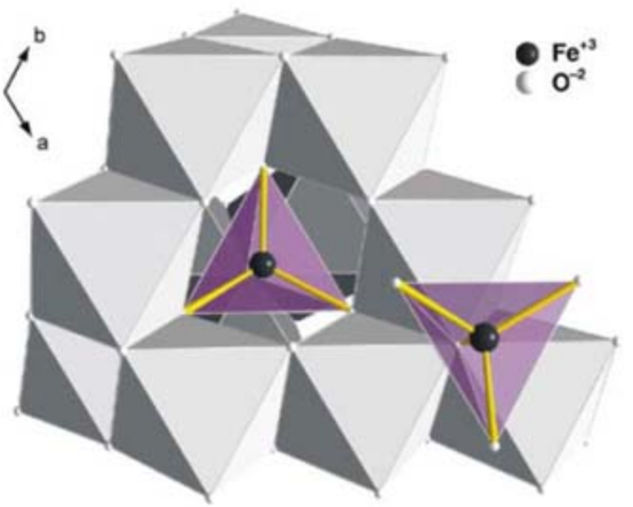

Fig. 3. Polyhedral representation of the ideal ferrihydrite structure viewed along the *c* axis. The central FeO_4 tetrahedra are surrounded by 12 FeO_6 octahedra.

References and Notes

1. D. G. Rancourt *et al.*, *Am. Mineral.* **86**, 834 (2001).
2. U. Schwertmann, L. Carlson, E. Murad, *Clays Clay Miner.* **35**, 297 (1987).
3. G. P. Huffman *et al.*, *Energy Fuels* **7**, 285 (1993).
4. P. A. Riveros, J. E. Dutrizac, P. Spencer, *Can. Metall. Q.* **40**, 395 (2001).
5. R. M. Cornell, U. Schwertmann, *The Iron Oxides: Structure, Properties, Reactions, Occurrence and Uses* (VCH, Weinheim, Germany, 1996).
6. A. Lewin, G. R. Moore, N. E. Le Brun, *Dalton Trans.* **2005**, 3597 (2005).
7. V. A. Drits, B. A. Sakharov, A. L. Salyn, A. Manceau, *Clay Miner.* **28**, 185 (1993).
8. D. E. Janney, J. M. Cowley, P. R. Buseck, *Am. Mineral.* **85**, 1180 (2000).
9. D. E. Janney, J. M. Cowley, P. R. Buseck, *Am. Mineral.* **86**, 327 (2001).
10. E. Jansen, A. Kyek, W. Schafer, U. Schwertmann, *Appl. Phys. Mater. Sci. Process.* **74**, S1004 (2002).
11. J. L. Jambor, J. E. Dutrizac, *Chem. Rev.* **98**, 2549 (1998).
12. F. M. Michel *et al.*, *Chem. Mater.* **19**, 1489 (2007).
13. S. J. L. Billinge, M. G. Kanatzidis, *Chem. Commun.* **7**, 749 (2004).
14. T. Egami, S. J. L. Billinge, *Underneath the Bragg Peaks: Structural Analysis of Complex Materials*, vol. 7 of *Pergamon Materials Series*, R. W. Cahn, Ed. (Elsevier, Oxford, 2003).
15. S. L. Hwang, P. Y. Shen, H. T. Chu, T. F. Yui, *Int. Geol. Rev.* **48**, 754 (2006).
16. K. Kodama, S. Iikubo, T. Taguchi, S. Shamoto, *Acta Crystallogr. A* **62**, 444 (2006).

17. G. Paglia, E. S. Bozin, S. J. L. Billinge, *Chem. Mater.* **18**, 3242 (2006).
18. W. H. Casey, *Chem. Rev.* **106**, 1 (2006).
19. I. Joumard *et al.*, *Phys. Rev. B* **74**, 205411 (2006).
20. G. W. Brady *et al.*, *Biochemistry* **7**, 2185 (1968).
21. R. A. Eggleton, R. W. Fitzpatrick, *Clays Clay Miner.* **36**, 111 (1988).
22. Q. A. Pankhurst, R. J. Pollard, *Clays Clay Miner.* **40**, 268 (1992).
23. Y. Pan *et al.*, *Micron* **37**, 403 (2006).
24. Y. Pan, Ph.D. thesis, University of Leeds, Leeds, UK (2007).
25. Y. Guyodo *et al.*, *Physics of the Earth and Planetary Interiors* **154**, 222 (2006).
26. E. Murad, U. Schwertmann, *Am. Mineral.* **65**, 1044 (1980).
27. J. van Slageren *et al.*, *Phys. Rev. B* **73**, 014422 (2006).
28. P. Juhas, D. M. Cherba, P. M. Duxbury, W. F. Punch, S. J. L. Billinge, *Nature* **440**, 655 (2006).
29. V. Petkov *et al.*, *Phys. Rev. B* **65**, 092105 (2002).
30. B. Gilbert, F. Huang, H. Z. Zhang, G. A. Waychunas, J. F. Banfield, *Science* **305**, 651 (2004).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/1142525/DC1
SOM Text
Fig. S1
Tables S1 and S2
Crystallographic Information Files
References

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Deformation of $(Mg,Fe)SiO_3$ Post-Perovskite and D″ Anisotropy

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Polycrystalline $(Mg_{0.9},Fe_{0.1})SiO_3$ post-perovskite was plastically deformed in the diamond anvil cell between 145 and 157 gigapascals. The lattice-preferred orientations obtained in the sample suggest that slip on planes near (100) and (110) dominate plastic deformation under these conditions. Assuming similar behavior at lower mantle conditions, we simulated plastic strains and the contribution of post-perovskite to anisotropy in the D″ region at the Earth core-mantle boundary using numerical convection and viscoplastic polycrystal plasticity models. We find a significant depth dependence of the anisotropy that only develops near and beyond the turning point of a downwelling slab. Our calculated anisotropies are strongly dependent on the choice of elastic moduli and remain hard to reconcile with seismic observations.

Seismological observations of the lowermost mantle (the D″ region) have revealed a region of great complexity distinct from the overlying deep mantle (*1*, *2*). Unlike the bulk of the lower mantle, the core-mantle boundary (CMB) includes large-scale regions with apparent seismic anisotropy (*3*). It has been suggested that this anisotropy could reflect lattice-preferred orientation (LPO) of minerals (*4*) or alignment of structural elements, including layers of melt (*5*, *6*). A number of lines of evidence now suggest that the transition from a perovskite (Pv) to a post-perovskite (pPv) phase in $(Mg,Fe)SiO_3$ (*7*, *8*) could explain important properties of D″ (*9*–*13*). However, the influence of this phase transition on our understanding of D″ anisotropy remains ambiguous. In this paper, we present multiscale modeling of deformation-induced anisotropy from $(Mg,Fe)SiO_3$-pPv in D″. This work is a combination of high-pressure deformation experiments on $(Mg,Fe)SiO_3$-pPv and numerical modeling of convection using polycrystal plasticity to predict strain and anisotropy in D″.

We deformed a sample of polycrystalline $(Mg_{0.9},Fe_{0.1})SiO_3$-pPv plastically in the diamond anvil cell in compression between 145 and 157 GPa and observed the evolution of LPO in situ using angle dispersive radial x-ray diffraction (fig. S1) at the High-Pressure Collaborative Access Team (HPCAT) of the Advanced Photon Source (beamline 16-ID-B). Starting material was a powder of natural orthopyroxene (*14*) mixed with 10 weight percent Pt powder that served as a laser absorber. The sample was initially compressed to high pressure, at which we could not observe coherent diffraction from within the sample, and then converted into the pPv phase by laser heating at different sample positions at a temperature of 1700 K for 20 min and 2000 K for ~15 min. After the phase transformation, pressure and differential stress in the sample were 145 and 7.2 GPa, respectively. They were then increased in two steps to 157 and 8.5 GPa over the course of 30 hours. At every step, we collected radial diffraction patterns to evaluate the pressure, stress, and LPO in the sample (*14*) (table S1).

The diffraction images show substantial variations of diffraction peak positions and intensities with orientation relative to the compression direction that can be used to estimate stress and deduce LPO (*14*) (Fig. 1). For instance, we observed that the diffraction intensity in the compression direction is minimal for 004 and 022, whereas it is maximal for 113 and 132. The texture we obtain (*14*) is represented in Fig. 2. In contrast with low-temperature and lower-pressure observations on the Mn_2O_3 (*15*) and $CaIrO_3$ (*16*) pPv analogs, we observed LPO compatible with previous observations on a $MgGeO_3$-pPv analog deformed under similar conditions (*17*) with a clear minimum at (010) and (001). Those LPO are formed immediately upon synthesizing and heating $(Mg,Fe)SiO_3$-pPv at high pressure. Minima observed at (010) and (001) preclude slip on (001) and (010) planes, and a comparison of observed textures and results from viscoplastic self-consistent (VPSC) polycrystal plasticity simulations (*18*) indicate that the deformation is likely dominated by slip on planes such as (100) or (110), in agreement with results of first-principles modeling of stacking fault energetics and shear elastic constants SiO_3 (*19*) but in disagreement with first-principles modeling of dislocation cores based on the Peierls model that suggest [001](010) as the easiest slip system (*20*).

There are limitations in our experiment: time scale, grain size, strain, temperature, and deviatoric stresses are quite different from those in D″. Moreover, LPOs are formed immediately upon synthesizing and heating the pPv phase and do not evolve greatly upon further compression. However, assuming that (100) and (110) slip also applies to $(Mg,Fe)SiO_3$-pPv under deep mantle conditions, we simulated the development of LPO in $(Mg,Fe)SiO_3$-pPv in D″ combining geodynamic information about macroscopic deformation and the microscopic deformation mechanisms found in the experiment (table S2). Deformation in D″ can be quite complex because it is coupled to larger-scale mantle-wide convective processes. Therefore, we performed our modeling using the entire mantle domain. We used the numerical convection code Citcom (*21*) with the addition of Lagrangian tracers to obtain a proper estimation of the deformational characteristics of

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mantle strain expected in D″ and tracked deformation in tracers along several streamlines by computing the left-stretch tensor at each step (*14*, *22*, *23*). The two-dimensional (2D) convection calculation employed a Rayleigh number of 10^7, stress-free boundaries, a temperature-dependent rheology, and a viscosity jump of a factor of 50 across the 660-km phase transition (*14*). The formation of a rigid lid was inhibited by imposing a maximum allowable viscosity for the uppermost portion of the model, allowing strong slabs to form. As is typical for geodynamical modeling, we employed the Boussinesq approximation to minimize the number of free parameters. This approximation excludes the effects of compressibility, viscous dissipation, adiabatic heating/cooling, and buoyancy effects due to phase transitions, including the Pv-pPv phase transition. We would expect that our predicted strain values would be slightly modified with the inclusion of these smaller-order physical processes; however, given the uncertainties in the model parameters, we predict that the difference would be minor, particularly for this study.

We began tracking deformation in slab regions at about 290 km above the CMB, corresponding to an approximate depth at which the Pv-to-pPv phase transition is expected to occur. The general trend of strain appears to be similar for most tracers and is characterized by horizontal stretching as slab material impinges upon the CMB (Fig. S4). After investigating several streamlines and observing similar trends, we concentrated on one particular streamline for use in polycrystal plasticity models (*18*). Accumulated strains along a streamline are very large and, assuming that all this strain is accommodated by dislocation glide, polycrystal plasticity simulations would predict very sharp textures, close to a single crystal. This is clearly not realistic. At high temperatures, strain may be partially accommodated by climb, boundary diffusion, and dynamic recrystallization that may significantly weaken texture development. Furthermore, secondary phases may be present. Thus, after several tests (*14*), we found that a reasonable assumption is that 10% of the plastic strain recorded by the tracer is accommodated by dislocation glide in pPv and the rest by mechanisms that do not produce preferred orientation. Using the VPSC model, we simulated the LPO evolution of an aggregate of 2000 grains at each time step of the convection model. Most of the strain (Fig. 3 and fig. S5) occurs as the aggregate reaches the CMB and flows parallel to it. This configuration is very similar to a combination of pure and simple shear parallel along the freeslip surface of the CMB. As the tracer descends into D″, we observe very little development of LPO (Fig. 3A). Texture develops rapidly between steps 1000 (Fig. 3A) and 2000 (Fig. 3B) as the tracer turns at the CMB. The texture strengthens and evolves only moderately as the particle moves along the CMB (up to step 5000) (Fig. 3C) and is later modified during upwelling (Fig. 3D).

We obtained an estimate of expected anisotropies in the D″ by averaging the single-crystal elastic tensors as a function of crystallographic orientation. From the aggregate elastic tensor, we then calculated seismic velocities in different directions. First-principles calculations provide single-crystal elastic moduli for $MgSiO_3$-pPv at high pressure and high temperature (*24*, *25*) (table S3).

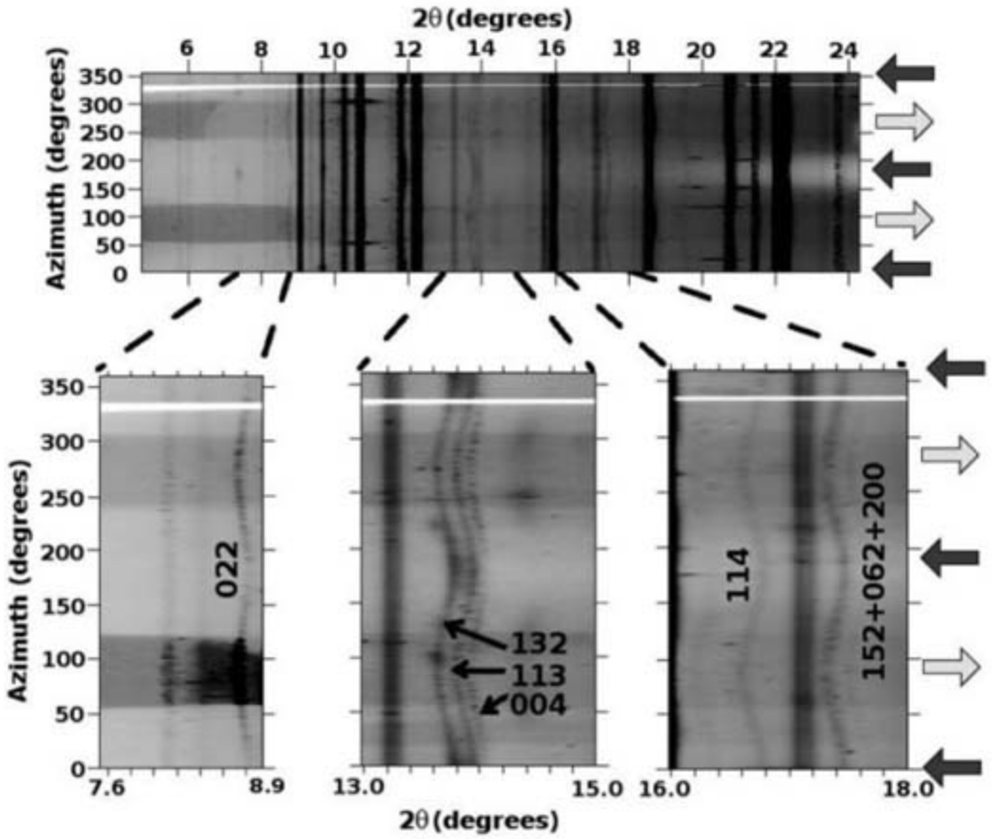

Fig. 1. Unrolled diffraction image of $(Mg,Fe)SiO_3$-pPv measured in radial diffraction, in situ, at 145 GPa. The directions of maximum and minimum stress are indicated by the black and gray arrows on the right, respectively. LPO and differential stress are deduced from the variations of diffraction intensity and peak position with orientation. Miller indices of the diffraction lines from the $(Mg,Fe)SiO_3$-pPv sample actually used in the analysis are labeled on the figure. Diffraction lines with no evidence of stress (straight lines) are from the gasket.

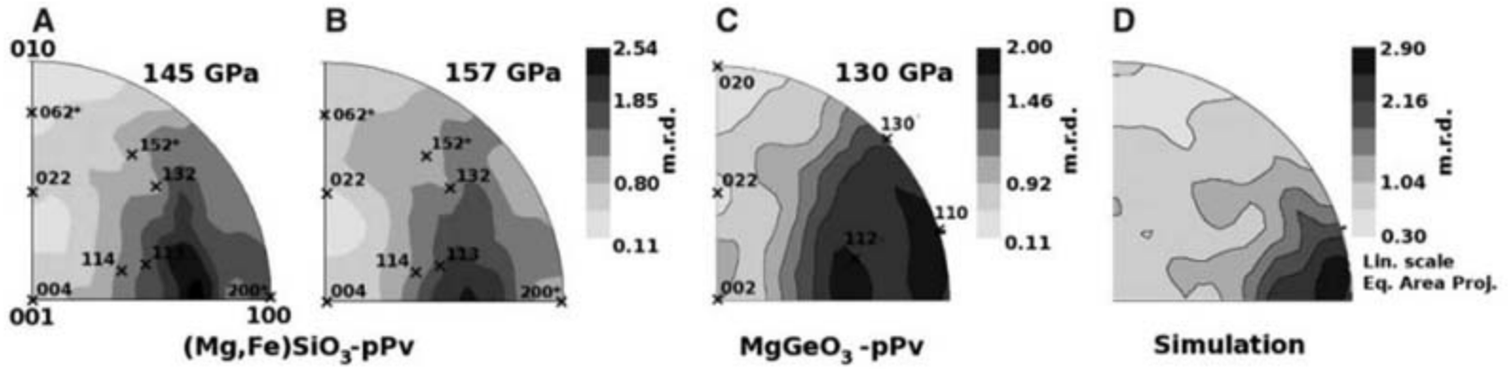

Fig. 2. Inverse pole figure showing the preferred orientation pattern in $(Mg,Fe)SiO_3$-pPv in compression measured (**A**) at 145 GPa just after converting the material to the pPv phase, (**B**) at 157 GPa, (**C**) in $MgGeO_3$ pPv at 130 GPa (*17*), and (**D**) simulated after 20% compressive strain with models that favor slip on (100) and (110). Equal-area projection is used, and linear contours express pole densities in multiples of a random distribution. Reflections used for inverting the orientation distribution function are indicated in the experimental inverse pole figures. The 152, 062, and 200 peaks of $(Mg,Fe)SiO_3$-pPv overlap and are not well resolved.

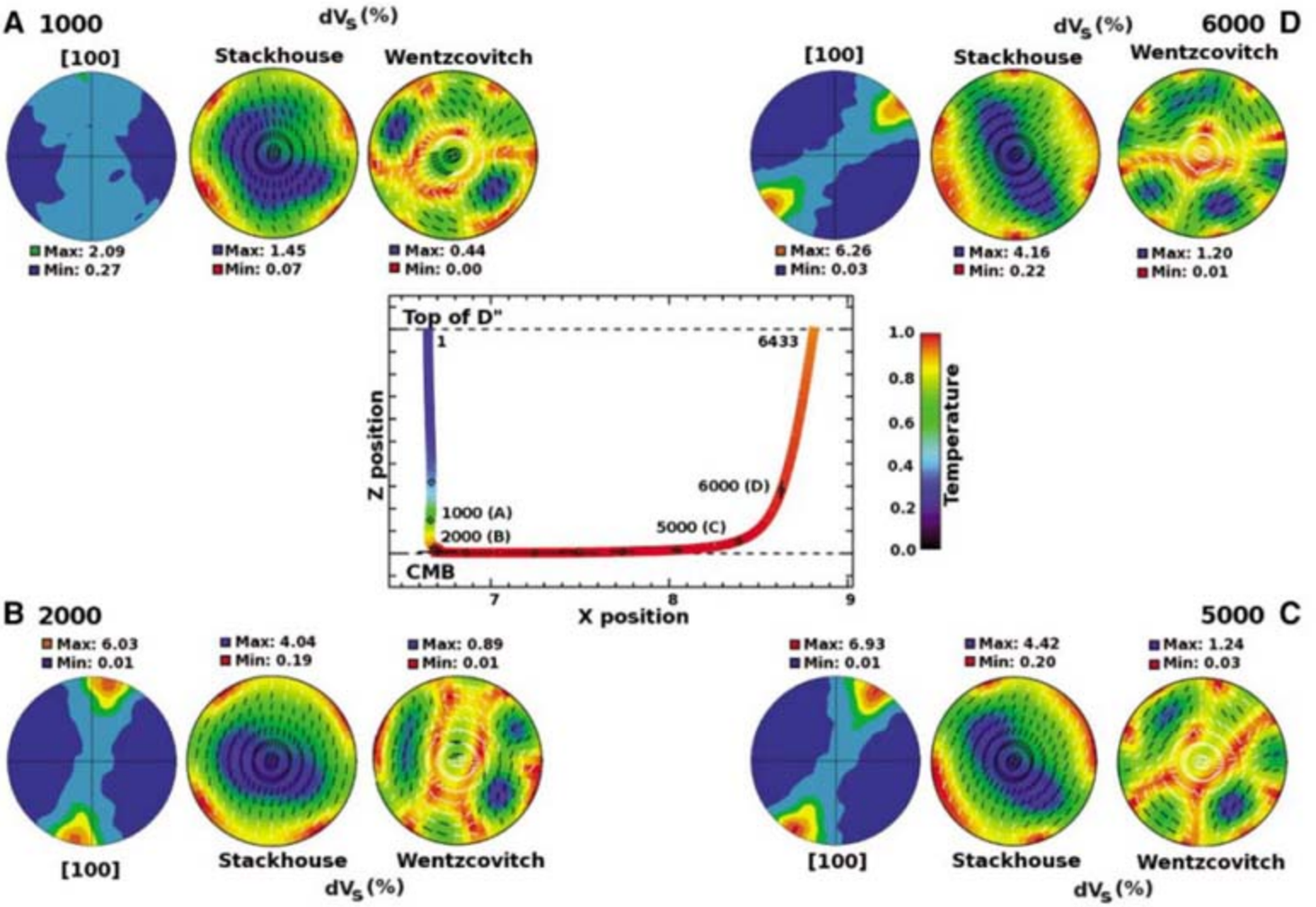

Fig. 3. Modeled temperature, strain, texture, and shear wave splitting from silicate post-perovskite in D″. The central figure illustrates the evolution of temperature and strain along a streamline. Temperatures are normalized so that $T = 0$ at Earth's surface and $T = 1$ at the CMB, strains are indicated by the black lines representing the evolution of maximum and minimum stretch of the Lagrangian particle for every 500 time steps, and numbers are time-step numbers. Panels (**A**) to (**D**) present the modeled 3D [100] orientations, shear wave splitting dV_S, and fastest shear wave polarizations at time steps 1000, 2000, 5000, and 6000, respectively. Shear wave splitting was calculated using the elastic moduli of Stackhouse *et al.* (*24*) and Wentzcovitch *et al.* (*25*). Linear scale, equal area projection. Contours for the [100] and dV_S pole figures are expressed in multiples of a random distribution and percentage, respectively. Black and white lines (for low and high anisotropies, respectively) indicate the direction of polarization of the fast shear wave.

The results of the two calculations differ significantly, and we decided to include both in our analysis (figs. S8 to S10). As the tracer plunges into the D″ layer, we observe very little anisotropy. It develops rapidly between steps 1000 and 2000 (Fig. 3, A and B), as the tracer reaches the CMB. At step 5000 (Fig. 3C), before entering the upwelling, shear wave splitting reaches 4.42% and 1.24% with the elastic moduli of Stackhouse *et al.* (*24*) and Wentzcovitch *et al.* (*25*), respectively.

Most seismic observations of shear wave polarization anisotropy in D″ involve delays of vertically polarized *S* wave components (*SV*) relative to horizontally polarized *S* wave components (*SH*) for paths that graze horizontally through the D″ region (*26*). However, other studies show tilted transverse anisotropy (*27*, *28*) or local variations of fast polarization directions (*29*–*31*). Our predictions of shear wave polarization anisotropies depend strongly on the choice of elastic moduli. Using both models, we found a significant depth dependence of the anisotropy that only develops near and beyond the turning point of a downwelling slab (Fig. 3 and figs. S8 to S10). In a slablike environment, we also found that the anisotropy for waves propagating parallel to the CMB should produce loosely symmetric patterns. Using the elastic moduli of Stackhouse *et al.* (*24*), we predict a maximum anisotropy of 4% perpendicular to the direction of flow, with the direction of fast polarization ranging from 60° to 90° ($V_{SH} < V_{SV}$). Using the elastic moduli of Wentzcovitch *et al.* (*25*), we obtain lower values of anisotropy with dV_S ranging between 0.5 and 0.6%, with a fast polarization near 0° ($V_{SH} > V_{SV}$) for waves traveling perpendicular to the direction of flow. Waves traveling parallel to the direction of flow would show varying anisotropies with very little anisotropy near the downwelling and up to 1.2% before upwelling, again with a fast polarization near 0° ($V_{SH} > V_{SV}$).

Predictions using the elastic moduli of Stackhouse *et al.* (*24*) systematically imply $V_{SH} < V_{SV}$ and are inconsistent with most seismic measurements that find either $V_{SH} > V_{SV}$ or locally varying fast polarization directions. Using the elastic moduli of Wentzcovitch *et al.* (*25*), we obtain complex patterns of anisotropies with lower amplitude than observed seismically. Although many characteristics of D″ are consistent with the properties of post-perovskite, other phases or structural mechanisms (e.g., layering) may be necessary to explain the seismic anisotropy of the region. Our study shows how ultrahigh pressure experiments on silicate post-perovskites combined with microscale (plasticity) and macroscale (mantle flow) modeling provide the means to test our understanding of deformation behavior at the base of Earth's mantle.

References and Notes

1. T. Lay, Q. Williams, E. J. Garnero, *Nature* **392**, 461 (1998).
2. R. D. van der Hilst *et al.*, *Science* **315**, 1813 (2007).
3. M. Panning, B. Romanowicz, *Science* **303**, 351 (2004).
4. A. K. McNamara, P. E. van Keken, S. I. Karato, *Nature* **416**, 310 (2002).
5. Q. Williams, E. J. Garnero, *Science* **273**, 1528 (1996).
6. J. M. Kendall, in *The Core-Mantle Boundary Region*, M. Gurnis, M. E. Wysession, E. Knittle, B. A. Buffet, Eds.

(American Geophysical Union, Washington, DC, 1998), pp. 97–118.
7. M. Murakami, K. Hirose, K. Kawamura, N. Sata, Y. Ohishi, *Science* **304**, 855 (2004).
8. A. R. Oganov, S. Ono, *Nature* **430**, 445 (2004).
9. D. Helmberger, T. Lay, S. Ni, M. Gurnis, *Proc. Nat. Acad. Sci. U.S.A.* **102**, 17257 (2005).
10. J. Wookey, S. Stackhouse, J. Kendall, J. Brodholt, G. D. Price, *Nature* **438**, 1004 (2005).
11. K. Hirose, *Rev. Geophys.* **44**, RG3001 (2006).
12. W. L. Mao *et al.*, *Science* **312**, 564 (2006).
13. T. Lay, J. Hernlund, E. J. Garnero, M. S. Thorne, *Science* **314**, 1272 (2006).
14. Materials and methods are available as supporting material on *Science* Online.
15. J. Santillán, S. Shim, G. Shen, V. Prakapenka, *Geophys. Res. Lett.* **33**, L15307 (2006).
16. D. Yamazaki, T. Yoshino, H. Ohfuji, J. Ando, A. Yoneda, *Earth Planet. Sci. Lett.* **252**, 372 (2006).
17. S. Merkel *et al.*, *Science* **311**, 644 (2006).
18. R. A. Lebensohn, C. N. Tomé, *Acta Metal. Mater.* **41**, 2611 (1993).
19. A. R. Oganov, R. Martoňák, A. Laio, P. Raiteri, M. Parrinello, *Nature* **438**, 1142 (2005).
20. P. Carrez, D. Ferré, P. Cordier, *Nature* **446**, 68 (2007).
21. L. Moresi, M. Gurnis, *Earth Planet. Sci. Lett.* **138**, 15 (1996).
22. A. K. McNamara, P. E. van Keken, S.-I. Karato, *J. Geophys. Res.* **108**, 2230 (2003).
23. A. K. McNamara, S. Zhong, *Earth Planet. Sci. Lett.* **222**, 485 (2004).
24. S. Stackhouse, J. P. Brodholt, J. Wookey, J.-M. Kendall, G. D. Price, *Earth Planet. Sci. Lett.* **230**, 1 (2005).
25. R. Wentzcovitch, T. Tsuchiya, J. Tsuchiya, *Proc. Nat. Acad. Sci. U.S.A.* **103**, 543 (2006).
26. M. Moore, E. J. Garnero, T. Lay, Q. Williams, *J. Geophys. Res.* **109**, B02319 (2004).
27. E. J. Garnero, V. Maupin, T. Lay, M. J. Fouch, *Science* **306**, 259 (2004).
28. J. Wookey, J. M. Kendall, G. Rümpker, *Geophys. J. Int.* **161**, 829 (2005).
29. S. A. Russell, T. Lay, E. J. Garnero, *Nature* **396**, 255 (1998).
30. S. A. Russell, T. Lay, E. J. Garnero, *J. Geophys. Res.* **104**, 13,183 (1999).
31. J. M. Rokosky, T. Lay, E. J. Garnero, *Earth Planet. Sci. Lett.* **248**, 411 (2006).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S10
Tables S1 to S3
References

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Weak Northern and Strong Tropical Land Carbon Uptake from Vertical Profiles of Atmospheric CO_2

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Measurements of midday vertical atmospheric CO_2 distributions reveal annual-mean vertical CO_2 gradients that are inconsistent with atmospheric models that estimate a large transfer of terrestrial carbon from tropical to northern latitudes. The three models that most closely reproduce the observed annual-mean vertical CO_2 gradients estimate weaker northern uptake of −1.5 petagrams of carbon per year (Pg C year^{-1}) and weaker tropical emission of +0.1 Pg C year^{-1} compared with previous consensus estimates of −2.4 and +1.8 Pg C year^{-1}, respectively. This suggests that northern terrestrial uptake of industrial CO_2 emissions plays a smaller role than previously thought and that, after subtracting land-use emissions, tropical ecosystems may currently be strong sinks for CO_2.

Our ability to diagnose the fate of anthropogenic carbon emissions depends critically on interpreting spatial and temporal gradients of atmospheric CO_2 concentrations (*1*). Studies using global atmospheric transport models to infer surface fluxes from boundary-layer CO_2 concentration observations have generally estimated the northern mid-latitudes to be a sink of approximately 2 to 3.5 Pg C year^{-1} (*2*–*5*). Analyses of surface ocean partial pressure of CO_2 (*2*), atmospheric carbon isotope (*6*), and atmospheric oxygen (*7*) measurements have further indicated that most of this northern sink must reside on land. Tropical fluxes are not well constrained by the atmospheric observing network, but global mass-balance requirements have led to estimates of strong (1 to 2 Pg C year^{-1}) tropical carbon sources (*4*, *5*). Attribution of the Northern Hemisphere terrestrial carbon sink (*8*–*13*) and reconciliation of estimates of land-use carbon emissions and intact forest carbon uptake in the tropics (*14*–*19*) have motivated considerable research, but these fluxes remain quantitatively uncertain. The full range of results in a recent inverse model comparison study (*5*), and in independent studies (*3*, *20*, *21*), spans budgets with northern terrestrial uptake of 0.5 to 4 Pg C year^{-1}, and tropical terrestrial emissions of −1 to +4 Pg C year^{-1}. Here, we analyzed observations of the vertical distribution of CO_2 in the atmosphere that provide new constraints on the latitudinal distribution of carbon fluxes.

Previous inverse studies have used boundary-layer data almost exclusively. Flask samples from profiling aircraft have been collected and measured at a number of locations for up to several decades (*22*–*24*), but efforts to compile these observations from multiple institutions and to compare them with predictions of global models have been limited. Figure 1 shows average vertical profiles of atmospheric CO_2 derived from flask samples collected from aircraft during midday at 12 global locations (fig. S1), with records extending over periods from 4 to 27 years (table S1 and fig. S2) (*25*). These seasonal and annual-mean profiles reflect the combined influences of surface fluxes and atmospheric mixing. During the summer in the Northern Hemisphere, midday atmospheric CO_2 concentrations are generally lower near the surface than in the free troposphere, reflecting the greater impact of terrestrial photosynthesis over industrial emissions at this time. Sampling locations over or immediately downwind of continents show larger gradients than those over or downwind of ocean basins in response to stronger land-based fluxes, and higher-latitude locations show greater CO_2 drawdown at high altitude. Conversely, during the winter, respiration and fossil-fuel sources lead to elevated low-altitude atmospheric CO_2 concentrations at northern locations. The gradients are comparable in magnitude in both seasons, but the positive

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gradients persist for a greater portion of the year and the annual-mean gradients also show higher atmospheric CO_2 concentrations near the surface than aloft. We estimated average Northern Hemisphere profiles (thick black lines in Fig. 1) by combining records from 10 sites (*25*). We found average Northern Hemisphere midday differences between altitudes of 1 and 4 km of −2.2 parts per million (ppm) in summer, +2.6 ppm in winter, and +0.7 ppm in annual mean. The two Southern Hemisphere locations show relatively constant CO_2 profiles in all seasons, with slightly higher values in the free troposphere.

To assess the performance of global atmospheric transport models used in CO_2 inverse studies, we compared the model predictions to our observations. We sampled the post-inversion concentration fields from the 12 models participating in the Transcom 3 Level 2 seasonal inversion experiment (T3L2) (*5*) at the airborne sampling locations in Fig. 1 and then fit and averaged these model predictions in the same manner as the observations (*25*). The models reproduce the general features of depleted low-altitude CO_2 during the summer and enhanced low-altitude CO_2 during the winter, but with important systematic differences (Fig. 2). Most of the models have gradients that are too small in the summer (Fig. 2A), suggesting that these models ventilate too much of the CO_2 uptake signal at this time of year. In the winter, the models match the observed gradients on average but include cases where vertical mixing appears both underestimated and overestimated. The predicted Northern Hemisphere annual-mean midday gradients are considerably larger than observed (Fig. 2B) and represent a substantial bias in the models. This overprediction of the annual-mean vertical gradients is also apparent when comparing models and data at individual sampling locations and is most pronounced at sites over or downwind of continents (fig. S6). The offset between the mean of the models and the observations at high-altitude in the summer (Fig. 2A) appears to be related to lags in the timing of the hemispheric CO_2 drawdown and to the fact that the models were optimized to marine boundary-layer stations, whereas the profile sites include measurements over the continental interiors. We focused only on the vertical gradients, which respond more quickly than the column means and are largely independent of where the models were optimized.

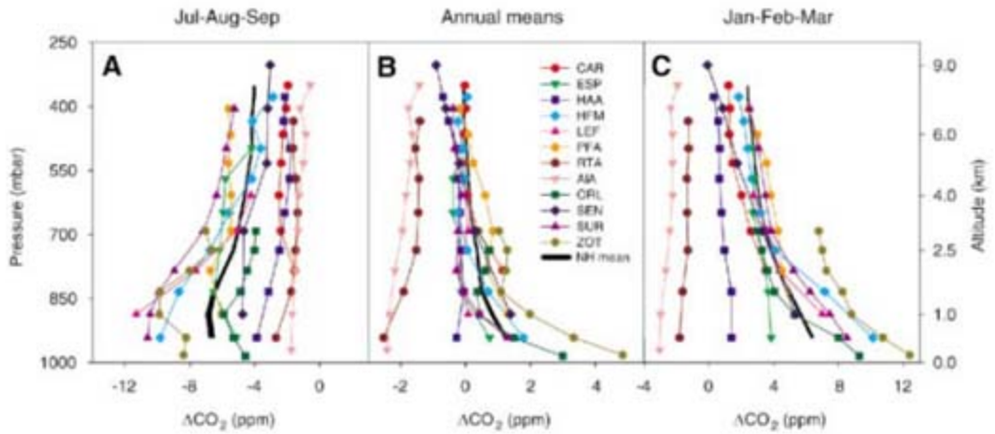

Fig. 1. Midday vertical CO_2 profiles measured at 12 global locations based on fits to samples binned by altitude and averaged over different seasonal intervals. Northern Hemisphere sites include Briggsdale, Colorado, United States (CAR); Estevan Point, British Columbia, Canada (ESP); Molokai Island, Hawaii, United States (HAA); Harvard Forest, Massachusetts, United States (HFM); Park Falls, Wisconsin, United States (LEF); Poker Flat, Alaska, United States (PFA); Orleans, France (ORL); Sendai/Fukuoka, Japan (SEN); Surgut, Russia (SUR); and Zotino, Russia (ZOT). Southern Hemisphere sites include Rarotonga, Cook Islands (RTA) and Bass Strait/Cape Grim, Australia (AIA). Profiles are averaged over Northern Hemisphere summer (**A**), all months (**B**), and Northern Hemisphere winter (**C**). A smoothed deseasonalized record from Mauna Loa has been subtracted from the observations at each site. Black lines in each panel represent Northern Hemisphere average profiles (center) and uncertainties (width) for the same times (*25*). The horizontal axis in (B) is zoomed by a factor of 2 relative to those in (A) and (C).

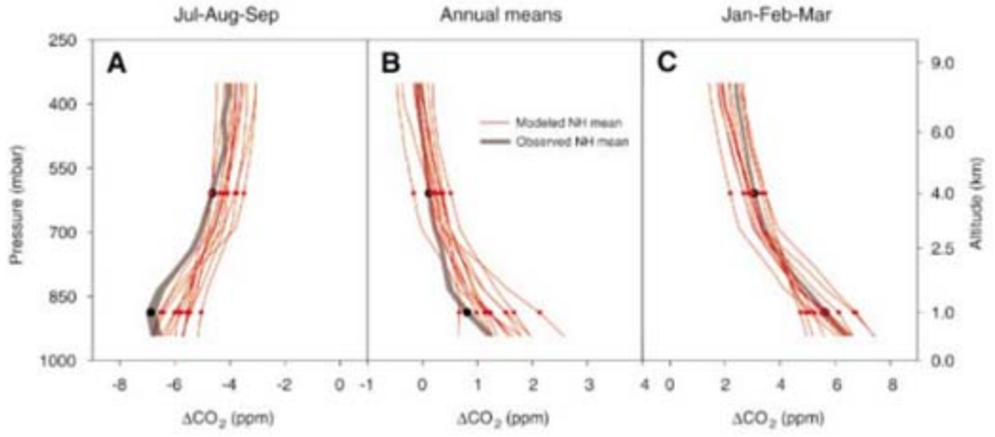

Fig. 2. (**A** to **C**) Observed Northern Hemisphere average profiles compared with predictions of the 12 T3L2 models over the same seasonal intervals as in Fig. 1. Gray lines indicate the observed average vertical CO_2 gradients (center) and uncertainties (width) from Fig. 1 (*25*). The model output was processed in the same way as the observations at each site before averaging (*25*). Symbols indicate 1- and 4-km values used for calculating the vertical gradients shown in Fig. 3. The horizontal axis in (B) is zoomed by a factor of 2 relative to those in (A) and (C).

Because the T3L2 models were primarily constrained by boundary-layer measurements, these post-inversion vertical gradients reflect the vertical mixing characteristics of the models [supporting online material (SOM) text]. Atmospheric mixing, surface CO_2 fluxes, and CO_2 spatial gradients are tightly linked in inverse calculations such that any biases in mixing, horizontal or vertical, will translate into biases in estimated fluxes. Figure 3 shows the impact of the range of vertical mixing behavior on northern and tropical land fluxes estimated using these models. Models that trap more CO_2 near the surface in the Northern Hemisphere during the winter require relatively weaker northern land emissions during this period to match surface observations, with a high degree of correlation (Fig. 3C). This vertical gradient-flux correlation is not as clear in the summer, probably because fossil-fuel burning and photosynthesis have opposing effects on concentration gradients, although there is a suggestion that models that ventilate summer uptake signals more efficiently require stronger northern land uptake to match the boundary-layer observations. These relationships are preserved when averaging over the annual cycle, and models with seasonal mixing characteristics that result in higher annually averaged CO_2 near the surface relative to aloft in the Northern Hemisphere estimate substantially greater annual-mean northern land uptake.

Because global CO_2 mass-balance must be maintained, and because the seasonally varying interaction of atmospheric mixing and terrestrial fluxes produces gradients primarily between northern and tropical latitudes, the models estimate compensating variations in tropical land fluxes in all three cases (Fig. 3). Models that estimate strong northern land uptake also estimate strong tropical land emissions. The tropical variations are larger and their correlations to the vertical gradients better than for the northern land fluxes, possibly because tropical fluxes are

less constrained by atmospheric CO_2 measurements and are consequently more susceptible to influence from model transport biases. These large across-model variations in northern and tropical land fluxes are not random, but are systematically related to how the models distribute CO_2 fluxes vertically in the Northern Hemisphere in different seasons.

The gray bars in Fig. 3 indicate the corresponding observed mean 1- to 4-km gradients and uncertainties (*25*) and reveal that most models overpredict the annual-mean midday vertical gradients. We considered a number of potential biases in comparing aircraft flask sample CO_2 measurements to model output. These include potential model biases related to diurnal flux variations and coarse grid resolution, and potential observation biases related to diurnal concentration variations, measurement representativeness, interannual variations, fair-weather flying conditions, and interlaboratory offsets (SOM text, tables S4 and S5, and figs. S8 and S9). Although the fair-weather bias can be as large as 1 ppm at individual sites, when averaging across the Northern Hemisphere, all of these potential biases appear to be smaller than 0.2 ppm or in the wrong direction to explain the model-observation differences shown in Figs. 2 and 3.

These differences suggest that an average taken across all models does not provide the most robust estimate of northern versus tropical flux partitioning. Furthermore, no single model captures both the seasonal and annual-mean observed gradients accurately (table S3). Because small seasonal flux errors of the same sign can combine to result in larger annual-mean flux biases, we used only annual-mean gradients to evaluate the models' annual-mean flux estimates. The three models closest to the annual-mean vertical gradients (models 4, 5, and C; tables S2 and S3) estimate average northern land uptake and tropical land emission of -1.5 ± 0.6 ($\pm$SD) and $+0.1 \pm 0.8$ Pg C year^{-1}, respectively. The T3L2 12-model average northern and tropical land results were considerably different at -2.4 ± 1.1 and $+1.8 \pm 1.7$ Pg C year^{-1}, respectively (*5*). Models 4, 5, and C have concentration biases in summer and winter that are substantial, but these errors offset rather than compound as they do for other models (table S3), which results in more accurate annual-mean gradients and consequently implies more accurate annual-mean flux estimates (SOM text).

Our results suggest less carbon uptake by northern land ecosystems than previously thought. Furthermore, because land-use changes in the tropics are thought to cause strong carbon emissions (*16*–*18*), our results imply strong carbon uptake in undisturbed ecosystems. These flux revisions are consistent with other lines of evidence and may help to resolve several long-standing conflicts in global carbon budgeting (*26*). Terrestrial ecosystem models and inventory studies have estimated northern terrestrial carbon uptake rates that are considerably weaker than suggested by the T3L2 study and other inverse models (*8*, *10*, *17*, *27*, *28*). In the tropics, theoretical reasons to expect strong carbon uptake fluxes in intact tropical forests (*29*) have been at odds with the strong emissions estimated in the T3L2 study. Tropical land carbon budgets are uncertain because of high spatial and interannual variability and a lack of comprehensive measurements (*30*, *31*), but a weak emission flux resulting from a relatively weaker deforestation source combined with a relatively stronger sink has support from bottom-up estimates (*14*–*16*, *18*, *19*, *32*). Notably, a repartitioning of terrestrial fluxes between northern and tropical regions as implied here does not conflict with independent ^{13}C and O_2/N_2 constraints on the global land-ocean flux partitioning.

A number of studies have stressed that because of the large differences seen between atmospheric inverse models, their estimated spatial distribution of annual-mean fluxes should be interpreted with great caution (*3*, *20*, *33*). Our analysis of the vertical distribution of atmospheric CO_2 suggests that these differences are systematic and open to validation. Other model properties, such as horizontal mixing aloft and seasonal timing of prior flux estimates, will have different effects on estimated fluxes and should also be investigated. The present airborne observing network is relatively sparse, and as more data become available our results may be refined. Also, we did not use interstation concentration differences in our analyses, but if interlaboratory calibration offsets are minimized, additional model tests may be possible. Future atmospheric inverse models with accurate seasonal mixing behavior will result in improved estimates of global carbon cycling. The continuation and expansion of airborne measurement programs for CO_2 and related tracers, and advances in coupled ecosystem-atmosphere modeling, including validation against discrete measurements, will greatly advance this goal.

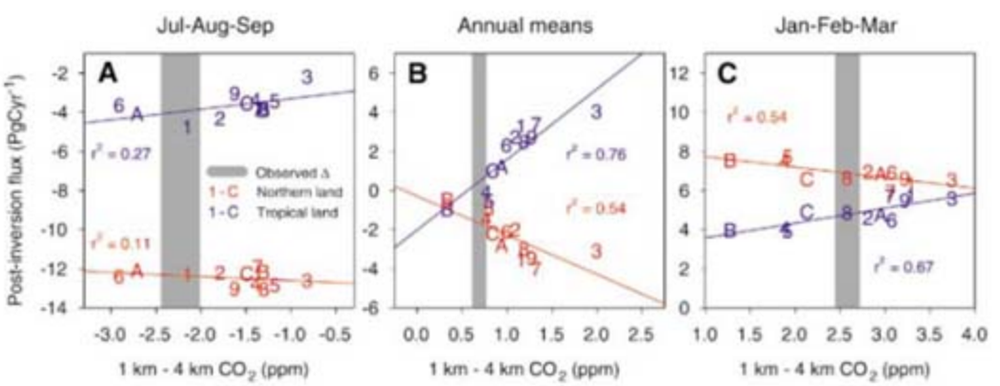

Fig. 3. Northern land and tropical land carbon fluxes for the 1992 to 1996 time period estimated by the 12 T3L2 models plotted as a function of the models' post-inversion predicted mean vertical CO_2 gradients for the same seasonal intervals as Fig. 1. The vertical axis in each plot represents the estimated fluxes for all northern land regions (red) and all tropical-land regions (blue) averaged over Northern Hemisphere summer (**A**), all months (**B**), and Northern Hemisphere winter (**C**). The horizontal axis represents the predicted Northern Hemisphere vertical CO_2 difference between 1- and 4-km altitude at these same times. The plotted numbers (1 to 9) and letters (A to C) correspond to the 12 models listed in table S2. Gray bars indicate the observed vertical CO_2 differences (center) from Fig. 2 and uncertainties (width) (*25*). The lines in each plot are linear least-squares fits to the modeled values.

References and Notes

1. A. S. Denning, I. Y. Fung, D. Randall, *Nature* **376**, 240 (1995).
2. P. P. Tans, I. Y. Fung, T. Takahashi, *Science* **247**, 1431 (1990).
3. P. Peylin, D. Baker, J. Sarmiento, P. Ciais, P. Bousquet, *J. Geophys. Res.* **107**, 4385 (2002).
4. K. R. Gurney *et al.*, *Nature* **415**, 626 (2002).
5. K. R. Gurney *et al.*, *Global Biogeochem. Cycles* **18**, GB1010 (2004).
6. P. Ciais, P. P. Tans, M. Trolier, J. W. C. White, R. J. Francey, *Science* **269**, 1098 (1995).
7. R. F. Keeling, S. C. Piper, M. Heimann, *Nature* **381**, 218 (1996).
8. P. Friedlingstein *et al.*, *Global Biogeochem. Cycles* **9**, 541 (1995).
9. R. B. Myneni, C. D. Keeling, C. J. Tucker, G. Asrar, R. R. Nemani, *Nature* **386**, 698 (1997).
10. D. Schimel *et al.*, *Science* **287**, 2004 (2000).
11. S. W. Pacala *et al.*, *Science* **292**, 2316 (2001).
12. C. C. Barford *et al.*, *Science* **294**, 1688 (2001).
13. W. De Vries, G. J. Reinds, P. Gundersen, H. Sterba, *Glob. Change Biol.* **12**, 1151 (2006).
14. J. Grace *et al.*, *Science* **270**, 778 (1995).
15. O. L. Phillips *et al.*, *Science* **282**, 439 (1998).
16. R. S. DeFries *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 14256 (2002).
17. R. A. Houghton, *Tellus* **55B**, 378 (2003).
18. F. Achard, H. D. Eva, P. Mayaux, H. Stibig, A. Belward, *Global Biogeochem. Cycles* **18**, GB2008 (2004).
19. S. L. Lewis, *Philos. Trans. R. Soc. London Ser. B* **361**, 195 (2006).
20. C. Rödenbeck, S. Houweling, M. Gloor, M. Heimann, *Tellus* **55B**, 488 (2003).
21. A. Jacobson, S. Mikaloff Fletcher, N. Gruber, J. Sarmiento, M. Gloor, *Global Biogeochem. Cycles* **21**, GB1020 (2007).
22. M. Tanaka, T. Nakazawa, S. Aoki, *J. Geophys. Res.* **88**, 1339 (1983).
23. R. J. Francey, L. P. Steele, R. L. Langenfelds, B. C. Pak, *J. Atmos. Sci.* **56**, 279 (1999).
24. GLOBALVIEW-CO_2, Cooperative Atmospheric Data Integration Project—Carbon Dioxide, CD-ROM, NOAA GMD, Boulder, CO (2006); available online via anonymous FTP to ftp.cmdl.noaa.gov, path: ccg/co2/GLOBALVIEW.
25. Materials and methods are available as supporting material on *Science* Online.
26. A. S. Denning, T. Takahashi, P. Friedlingstein, *Tellus* **51B**, 249 (1999).

27. R. K. Dixon *et al.*, *Science* **263**, 185 (1994).
28. I. A. Janssens *et al.*, *Science* **300**, 1538 (2003).
29. J. Lloyd, G. D. Farquhar, *Funct. Ecol.* **10**, 4 (1996).
30. S. R. Saleska *et al.*, *Science* **302**, 1554 (2003).
31. K. W. Holmes *et al.*, *Global Biogeochem. Cycles* **20**, GB3004 (2006).
32. A. D. McGuire *et al.*, *Global Biogeochem. Cycles* **15**, 183 (2001).
33. D. F. Baker *et al.*, *Global Biogeochem. Cycles* **20**, GB1002 (2006).
34. We thank the following T3L2 modelers for sharing their model output with us: R. M. Law, P. J. Rayner, D. Baker, Y.-H. Chen, I. Y. Fung, S. Houweling, J. John, T. Maki, S. Maksyutov, P. Peylin, M. Prather, B. C. Pak, and S. Taguchi. We thank A. Jacobson for helpful discussions. This work was supported by funding from the NSF, which sponsors the National Center for Atmospheric Research. The Transcom 3 experiment was made possible through support from the NSF (OCE-9900310), the National Oceanic and Atmospheric Administration (NA67RJ0152, Amend 30), and the International Geosphere Biosphere Program/Global Analysis, Interpretation, and Modeling Project. The Zotino measurements are supported by the European Union through the TCOS-Siberia contract (EVK2-CT-2001-00131) within the Fifth Framework Program. The Bass Strait/Cape Grim aircraft sampling program was supported by the Australian Bureau of Meteorology (1992–98), and the CSIRO Office of Space Science and Applications (1999–2000).

Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5832/1732/DC1
Materials and Methods
SOM Text
Figs. S1 to S9
Tables S1 to S5
References

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Saturation of the Southern Ocean CO_2 Sink Due to Recent Climate Change

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Based on observed atmospheric carbon dioxide (CO_2) concentration and an inverse method, we estimate that the Southern Ocean sink of CO_2 has weakened between 1981 and 2004 by 0.08 petagrams of carbon per year per decade relative to the trend expected from the large increase in atmospheric CO_2. We attribute this weakening to the observed increase in Southern Ocean winds resulting from human activities, which is projected to continue in the future. Consequences include a reduction of the efficiency of the Southern Ocean sink of CO_2 in the short term (about 25 years) and possibly a higher level of stabilization of atmospheric CO_2 on a multicentury time scale.

Atmospheric CO_2 increases at only half the rate of human-induced CO_2 emissions because of the presence of large CO_2 sinks in the ocean and on land (*1*). The sinks are highly variable and sensitive to climate, yet they are poorly constrained by observations. In the ocean, only the large-scale variability and trends in the equatorial and North Pacific have been quantified (*2*, *3*). In other regions, time-series observations and repeated survey analysis exist, but their extrapolation at the scale of a basin is problematic because of the presence of large regional variability (*4*–*6*). Data are particularly sparse in the Southern Ocean, where the magnitude of the CO_2 sink is heavily disputed (*7*, *8*), its interannual variability is unknown, and its control on atmospheric CO_2 during glaciations is firmly established but still not understood or quantified (*9*, *10*).

We estimated the variability and trend in the CO_2 sink of the Southern Ocean during 1981 to 2004 using the spatiotemporal evolution of atmospheric CO_2 from up to 11 stations in the Southern Ocean and 40 stations worldwide (Fig. 1). We used an inverse method that estimates the CO_2 flux distribution and time variability that best matches the observed atmospheric CO_2 concentrations (*11*). The inversion uses observed atmospheric CO_2 concentrations from individual flask pair values and/or hourly values from in situ analyzers, as available (*12*) (fig. S1). The station set is kept constant throughout the inversion to minimize spurious variability from the inversion setup. We performed an identical inversion over four time periods using (i) 40 atmospheric stations for 1996 to 2004 (9 years), (ii) 25 atmospheric stations for 1991 to 2004 (14 years), (iii) 17 atmospheric stations for 1986 to 2004 time period (19 years), and (iv) 11 atmospheric stations for 1981 to 2004 (24 years). CO_2 fluxes and concentrations are linked by the atmospheric transport model TM3, with resolution of ~4° by 5° and 19 vertical levels, driven by interannual 6-hourly winds from National Centers for Environmental Prediction (NCEP) reanalysis (*13*). The a priori information does not involve any time-dependent elements. Although we focus on the Southern Ocean (south of 45°S), where the influence of the land is at its minimum, the inversion is global.

The variability in integrated sea-air CO_2 flux estimated by the inversions is ±0.14 Pg C year^{-1} (*14*) over the Southern ocean (Fig. 2). The

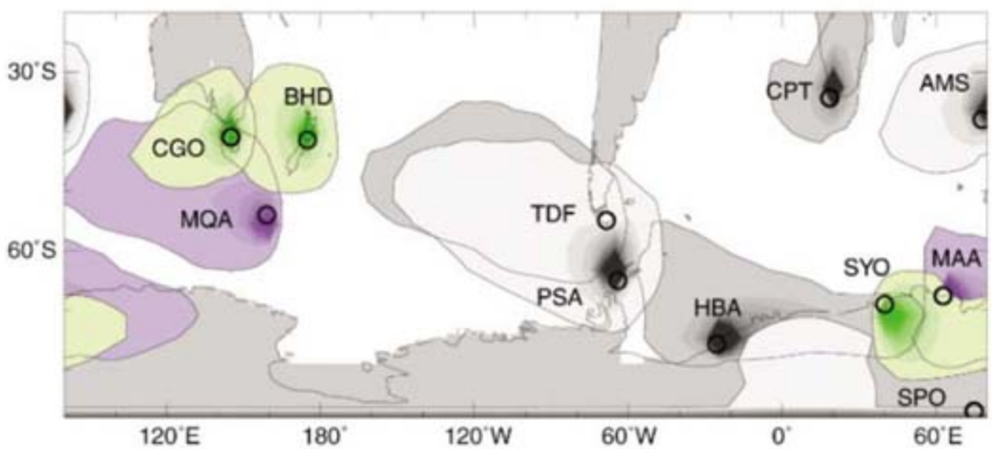

Fig. 1. Footprint of atmospheric CO_2 measurement stations. The footprint is defined here as the area where CO_2 fluxes of 0.2 mol/m^2 year^{-1} produce a concentration response of at least 1 ppm, on an annual average. The darkest shading shows the region with largest influence on a given station. Stations are Cape Grim (CGO; 40.7°S, 144.7°E); Macquarie Island (MQA; 54°S, 159°E); Baring Head (BHD; 41°S, 175°E); Tierra del Fuego (TDF; 54.9°S, 68.5°W); Palmer Station (PSA; 65.0°S, 64°W); Halley Bay (HBA; 75.7°S, 25.5°W); Cape Point (CPT; 34°S, 19°E); Syowa (SYO; 69°S, 39°E); Mawson (MAA; 68°S, 63°E); Amsterdam Island (AMS; 38°S, 78°E); and South Pole (SPO; 90.0°S). The color coding refers to the length of the station's record used, with light gray stations used since 1981, green stations since 1986, purple stations since 1991, and dark gray stations since 1996.

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amplitude of the CO_2 variability is about one-third of the amplitude of the flux variability associated with El Niño events in the equatorial Pacific (*2*) and ~10% of the variability observed in atmospheric CO_2 growth rate (*15*). The longer inversion reproduces most of the variability of the shorter, better constrained inversions.

The longer inversion further shows a decrease of the CO_2 sink in the Southern Ocean between 1981 and 2004 by 0.031 Pg C year^{-1} decade^{-1}. This decrease is significantly different at the 99.5% level (*16*) from the trend of –0.051 Pg C year^{-1} decade^{-1} in sea-air flux expected in response to the increase in atmospheric CO_2 alone (Fig. 2). We estimated the trend caused by increasing atmospheric CO_2 alone using two independent methods. First, we used a simple pulse response function, which we integrated in time using the observed atmospheric CO_2 growth rate as input (top red curve in Fig. 2) (*12*, *17*). This method takes into account the surface ocean equilibration with atmospheric CO_2 and the vertical transport of anthropogenic carbon into the ocean. Second, we used a full Ocean General Circulation Model (OGCM) coupled to a state-of-the-art biogeochemistry model [the Pelagic Interactions Scheme for Carbon and Ecosystem Studies version T (PISCES-T) model; bottom red curve in Fig. 2] (*12*), which we forced with atmospheric surface conditions from either years 1948, 1967, or 1979 repeatedly for all years (three separate simulations, only the 1967 result is plotted in Fig. 2), and with observed atmospheric CO_2 concentration. The pulse response and OGCM estimates have similar variability and a similar trend over the 1981 to 2004 time period (–0.051 and –0.057; –0.046, and –0.072 Pg C year^{-1} decade^{-1}, respectively) (Figs. 2 and 3).

The significant difference between the observed decrease of the CO_2 sink estimated by the inversion (0.03 Pg C year^{-1} decade^{-1}) and the expected increase due solely to rising atmospheric CO_2 (–0.05 Pg C year^{-1} decade^{-1}) indicates that there has been a relative weakening of the Southern Ocean CO_2 sink (0.08 Pg C year^{-1} per decade^{-1}) as a result of changes in other atmospheric forcing (winds, surface air temperature, and water fluxes). For comparison, the mean Southern Ocean CO_2 sink is estimated to be between 0.1 and 0.6 Pg C year^{-1} (table S1).

Inverse methods are sensitive to errors in the setup and transport model, in the data, and in the selection of the sites. We performed three series of sensitivity tests on the inversion results using the longest inversion. In the first series of tests, we assessed the robustness of the results to the choice of the most sensitive parameters of this inversion set up (*11*): (Is1 and Is2) We increased and decreased, respectively, the a priori standard deviation of the ocean and land CO_2 fluxes by a factor of four; (Is3) we increased the a priori standard deviation over the ocean and decreased that over land by a factor of 2 each; (Is4) we increased the spatial correlation scales by a factor of 2 (in latitude) and 4 (in longitude); and (Is5) we decreased the temporal correlation scale by a factor of 4. In the second series of tests, we assessed the robustness of the results with respect to transport errors by degrading the quality of the transport model: (It1) we reduced the resolution of the transport model by a factor of two; and (It2 and It3) we used the degraded model It1 and applied constant winds for years 1990 and 1995, respectively. In the third series of tests, we used the degraded model It1 and included further available data from (Id1) Baring Head, (Id2) Halley Bay, and (Id3) Cape Grim and Syowa, even though they are not available over all the period.

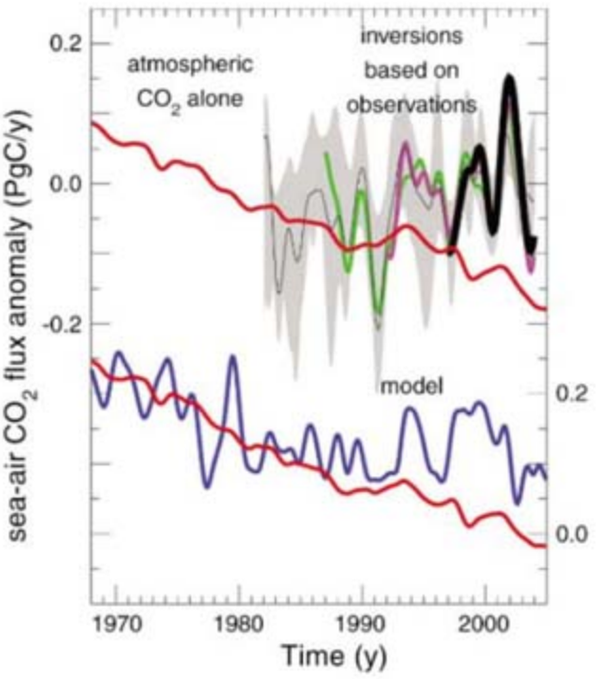

Fig. 2. Sea-air CO_2 flux anomalies in the Southern Ocean (Pg C year^{-1}). The contribution of atmospheric CO_2 alone (top red curve) is calculated based on observed atmospheric CO_2 concentration and a pulse response function that computes the ocean CO_2 uptake as a function of time (*12*, *17*). The estimates based on observations use an inverse model of atmospheric CO_2. Inversions over four time scales are shown starting in 1981 (thin black, 11 sites), 1986 (green, 17 sites), 1991 (purple, 25 sites), and 1996 (thick black, 40 sites). The gray shading encompasses results from all the sensitivity tests using the 11-site inversion. The lower panel shows results from a process model forced by (full red curve) the 1967 constant winds and fluxes and (blue curve) observed daily winds and fluxes from NCEP reanalysis. Sea-air CO_2 fluxes are integrated over 45°S to 90°S. Negative values indicate a flux of CO_2 from the atmosphere to the ocean, or a CO_2 sink into the ocean. Variability <1 year is removed using a Hanning filter for all time series. The 1995 to 2004 average was removed from all inversions (see table S1 for the spread in the mean). The mean of the atmospheric contribution is normalized to the inverted estimate for the 1981 to 1986 time period.

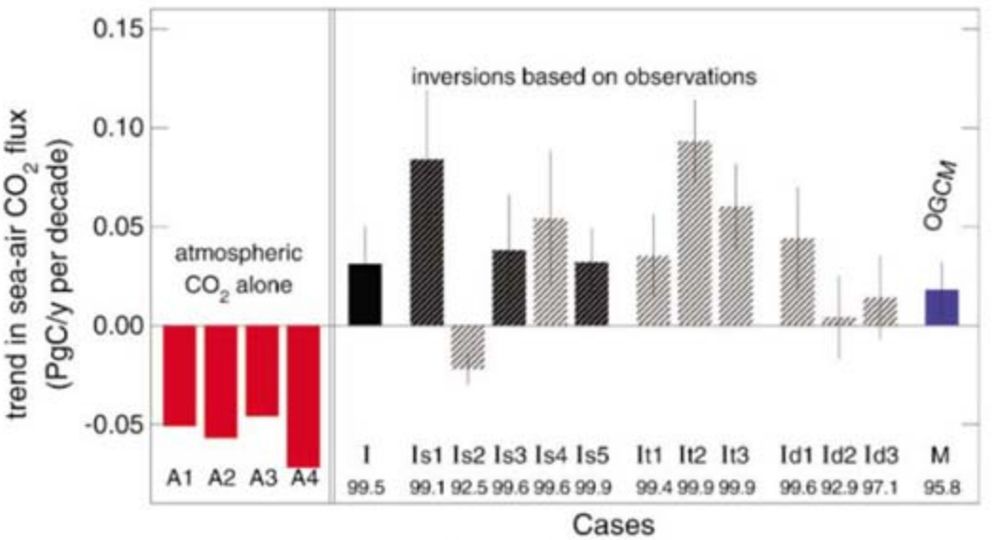

Fig. 3. Trend in the sea-air CO_2 flux (Pg C year^{-1} decade^{-1}). Cases A1 to A4 estimate the contribution of atmospheric CO_2 alone based on a pulse response model (A1) and an OGCM forced by constant winds and atmospheric fluxes (A2 to A4) from years 1948, 1967, and 1979, respectively. All inverse results are shown in black or gray. Case I is the standard inversion. Cases Is1 to Is5 are sensitivity tests to the model parameters. Cases It1 to It3 are sensitivity tests to the atmospheric transport model. Cases Id1 to Id3 are sensitivity tests to the selection of data. The sensitivity tests hatched dark produce the best match to the station data, whereas those hatched light produce the poorest match (*18*). Results of the process model using observed atmospheric forcing are shown in blue (M). Error bars for all cases indicate the amplitude of the interannual variability (± 1 SD). Significance of the departure between all the inversion cases and case A1 and between the model M and case A3 is also shown below each case (*16*).

In all the sensitivity tests, the trend in the CO_2 sink in the Southern Ocean is smaller than the trend caused by increasing atmospheric CO_2 alone (Fig. 3). Inversions I (standard inversion), Is1, Is3, and Is5 produced the best fit to observations (*18*). These inversions showed a decrease in CO_2 sink of 0.03 to 0.08 Pg C year^{-1} decade^{-1}, significantly different at the 99% level from the trend caused by atmospheric CO_2 alone (*16*). The inversions with the degraded transport model fit less well to the station data but still show a decrease in the CO_2 sink significantly different at the 99% level from the expected trend. The only sensitivity test that produces an increase in the CO_2 sink (Is2) also produces the worst fit to the observations (*18*). However, even this inversion produces a smaller increase in the CO_2 sink than that caused by atmospheric CO_2 alone, although the significance level is lower (92.5%).

We assessed the influence of the choice of stations further by comparing the trends in the long inversion with that of the 1986 to 2004 inversion, which uses 17 atmospheric stations instead of 11 (3 additional Southern Ocean stations). The trend in sea-air CO_2 flux in the two inversions for the overlapping period is similar, with 0.047 and 0.035 Pg C year^{-1} decade^{-1} for the 11-station and 17-station inversion, respectively, showing that the trend is correctly captured in the longer inversion.

The CO_2 flux variability from the longest inversion correlates with the Southern Annular Mode (SAM), an index of the dominant mode of atmospheric variability in the Southern Ocean. We use the SAM definition of Marshall (2003) (*19*), based on the difference in mean sea level pressure between 40°S and 65°S, which is entirely based on observations and fully independent of our inversion. The correlation of the monthly mean anomalies is small ($r = +0.22$) but significant at the 99% level (*16*, *18*). The positive correlation indicates that the ocean outgasses CO_2 compared with its mean state when the SAM is positive, i.e., when the winds are intensified south of 45°S (*20*), and suggests that wind-driven upwelling and associated ventilation of the subsurface waters rich in carbon dominates the variability in CO_2 flux (*18*).

To examine whether the results of the inversion can be traced back to physical processes, we estimated the variability and trend in CO_2 fluxes using the OGCM-PISCES-T model (*12*), now forced by daily wind stress and heat and water fluxes from the NCEP reanalyzed data for 1948 to 2004 (*13*), similar to (*21*). This process model reproduces similar patterns of variability in CO_2 flux as estimated by the inversion, with a smaller CO_2 sink (more positive sea-air CO_2 flux) during 1993 to 2003 compared with 1983 to 1993 and 2003 to 2004 (Fig. 2). The process model also produces a decrease in the CO_2 sink between 1981 and 2004 of 0.018 Pg C year^{-1} decade^{-1} (Fig. 3). The difference in sea-air CO_2 trend of +0.064 Pg C year^{-1} decade^{-1} between this simulation using observed atmospheric forcing and the simulation using constant forcing (–0.046 Pg C year^{-1} decade^{-1} using 1967 forcing) is entirely attributable to changes in ocean biogeochemistry caused by changes in surface atmospheric forcing. Thus, the process model attributes the decrease in CO_2 sink to an increase in outgassing of natural carbon (sea-air flux of +0.064 Pg C year^{-1} decade^{-1}) overcompensating the increase in the uptake of anthropogenic CO_2 (sea-air flux of –0.046 Pg C year^{-1} decade^{-1}), in agreement with results of the inversion based on observations.

We performed two additional simulations. First, the winds alone were kept constant at year 1967, but the heat and water fluxes were allowed to vary interannually. Results from this simulation show a trend in sea-air CO_2 flux that is close to the simulation where both winds and fluxes are kept constant (–0.034 compared with –0.046 Pg C year^{-1} decade^{-1}). Second, the winds were kept constant in the formulation of the gas exchange only but were allowed to vary in the physical model. The difference in trend with the variable gas exchange was very small (<3%). The results of the process model suggest that the changes in the CO_2 sink are dominated by the impact of changes in physical mixing and upwelling driven by changes in the winds on the natural carbon cycle in the ocean (*18*) (fig. S5), as suggested by the positive correlation between the inversion and the SAM. The process model also shows that the acidification of the surface ocean is accelerated by this process (*18*) (fig. S5).

On a multicentury time scale, results of simple models based on well-known carbon chemistry show that the ocean should take up 70 to 80% of all the anthropogenic CO_2 emitted to the atmosphere (*22*). This estimate takes into account changes in carbon chemistry but not the physical response of the natural carbon cycle to changes in atmospheric forcing. In the past, the marine carbon cycle has responded to circulation changes and cooling during glaciations by taking up enough carbon to lower atmospheric CO_2 by 80 to 100 parts per million (ppm) (*9*). Changes in Southern Ocean circulation resulting from changes in Southern Ocean winds (*23*) or buoyancy fluxes (*24*) have been identified as the dominant cause of atmospheric CO_2 changes (*9*, *10*, *25*). We showed that the Southern Ocean is responding to changes in winds over a much shorter time scale, thus suggesting that the long-term equilibration of atmospheric CO_2 in the future could occur at a level that is tens of ppm higher than that predicted when considering carbon chemistry alone.

Observations suggest that the trend in the Southern Ocean winds may be a consequence of the depletion of stratospheric ozone (*26*). Models suggest that part of the trend may also be caused by changes in surface temperature gradients resulting from global warming (*27*, *28*). Climate models project a continued intensification in the Southern Ocean winds throughout the 21st century if atmospheric CO_2 continues to increase (*28*). The ocean CO_2 sink will persist as long as atmospheric CO_2 increases, but (i) the fraction of the CO_2 emissions that the ocean is able to absorb may decrease if the observed intensification of the Southern Ocean winds continues in the future and (ii) the level at which atmospheric CO_2 will stabilize on a multicentury time scale may be higher if natural CO_2 is outgassed from the Southern Ocean.

References and Notes

1. I. C. Prentice *et al.*, in *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*, J. T. Houghton, Y. Ding, D. Griggs, M. Noguer, P. van der Linden, X. Dai, K. Maskell, C. Johnson, Eds. (Cambridge Univ. Press, Cambridge and New York, 2001), pp. 183–237.
2. R. A. Feely, R. Wanninkhof, T. Takahashi, P. Tans, *Nature* **398**, 597 (1999).
3. T. Takahashi, S. C. Sutherland, R. A. Feely, R. Wanninkhof, *Deep-Sea Res.* **111**, 10.1029/2005JC002074 (2006).
4. N. Gruber, C. D. Keeling, N. R. Bates, *Nature* **298**, 2374 (2002).
5. J. E. Dore, R. Lukas, D. W. Sadler, D. M. Karl, *Nature* **424**, 754 (2003).
6. A. Corbière, N. Metzl, G. Reverdin, C. Brunet, T. Takahashi, *Tellus*, doi:10.1111/j.1600-0889.2006.00232.x (2007).
7. T. Roy, P. Rayner, R. Francey, *Tellus* **55B**, 701 (2003).
8. N. Metzl, C. Brunet, A. Jabaud-Jan, A. Poisson, B. Schauer, *Deep-Sea Res. I* **53**, 1548, 10.1016/j.dsr.2006.07.006 (2006).
9. D. M. Sigman, E. Boyle, *Nature* **407**, 859 (2000).
10. K. E. Kohfeld, C. Le Quéré, S. P. Harrison, R. Anderson, *Science* **308**, 74 (2005).
11. C. Rödenbeck, Tech. Rep. 6, Max-Planck-Institute for Biogeochemistry, P.O. Box 100164, 07701 Jena, Germany, 2005. Available on www.bgc-jena.mpg.de/mpg/websiteBiogeochemie/Publikationen/Technical_Reports/tech_report6.pdf.
12. See full description in Supporting Online Material, including a comparison to other published inversions (fig. S2).
13. E. Kalnay *et al.*, *Bull. Am. Meteorol. Soc.* **77**, 437 (1996).
14. 0.28 Pg C year^{-1} for the peak-to-peak monthly anomalies in the longest inversion (1981 to 2004), equivalent to a standard deviation of 0.06 Pg C year^{-1}.
15. C. D. Keeling, T. P. Whorf, in *Trends: A Compendium of Data on Global Change* (Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN, 2005).
16. The statistical significance of the trend was estimated using the deseasonalized raw data and a 1000-member Monte Carlo ensemble from a noise model with the same standard deviation and lag-1 autocorrelation (*12*). The statistical significance only assesses the presence of a trend with respect to interannual variability and to random errors in the measurements. The significance of other errors and potential biases caused by the data coverage and inversion setup is assessed by the various sensitivity tests. The trend in the inversion result is not statistically different from zero, but from its expected value based on the increase in atmospheric CO_2 alone.
17. I. G. Enting, T. M. L. Wigley, M. Heimann, *CSIRO Aust. Div. Atmos. Res. Tech. Pap. No.* **31**, 1 (1994).
18. See further model results and evaluation in Supporting Online Material.
19. G. Marshall, *J. Clim.* **16**, 4134 (2003).
20. The increase in zonal winds is best documented by the observed increase in atmospheric pressure gradient between 40°S and 65°S (*19*). The NCEP reanalysis estimates an increase in zonal wind of ~1 to 2 m/s south of 45°S for a mean zonal wind of ~2 to 10 m/s.
21. P. Wetzel, A. Winguth, E. Maier-Reimer, *Global Biogeochem. Cycles* **19**, 10.1029/2004GB002339 (2005).
22. D. E. Archer, H. Kheshgi, E. Maier-Reimer, *Geophys. Res. Lett.* **24**, 405 (1997).
23. R. J. Toggweiler, J. L. Russel, S. Carlson, *Paleoceanogr.* **21**, 10.1029/2005PA001154 (2006).
24. A. J. Watson, A. C. Naveira Garabato, *Tellus* **58B**, 73 (2006).
25. E. W. Wolff *et al.*, *Nature* **440**, 491 (2006).
26. D. W. J. Thompson, S. Solomon, *Science* **296**, 895 (2002).

27. J. Fyfe, G. Boer, G. Flato, *Geophys. Res. Lett.* **26**, 1601 (1999).
28. D. T. Shindell, G. A. Schmidt, *Geophys. Res. Lett.* 10.1029/2004GL020724 (2004).
29. We thank S. Morimoto and everyone who participated in the collection of the atmospheric and oceanic CO_2 observations. The long-term observational project Ocean Indien Service d'Observations (OISO) is conducted on board the research vessel Marion-Dufresne and is supported, like the Amsterdam I. Réseau Atmosphérique de Mesure des Composés à Effet de Serre (RAMCES) observatory, by three Institutes in France, Institut National des Sciences de l'Univers (INSU), Institut Paul Emile Victor (IPEV), and Institut Pierre Simon Laplace (IPSL). We thank N. Gruber, N. Lovenduski, G. Marshall, H. Roscoe, and T. Mitchell for discussions; three anonymous reviewers for insightful comments; K. Rodgers, G. Madec, and the IPSL team for access to model codes; and the Deutsches Klimarechenzentrum (DKRZ) and Gesellschart für wissenschaftliche Datenverarbeitung mbH Göttingen (GWDG) centers for computer support. E.T.B. was partly supported by the EU project CARBOOCEAN (511176[GOCE]) and by the UK NERC/QUEST project Marquest (NE/C516128/1).

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Methods
Figs. S1 to S8
Tables S1
References

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Evolutionary Dynamics of Immune-Related Genes and Pathways in Disease-Vector Mosquitoes

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Mosquitoes are vectors of parasitic and viral diseases of immense importance for public health. The acquisition of the genome sequence of the yellow fever and Dengue vector, *Aedes aegypti* (*Aa*), has enabled a comparative phylogenomic analysis of the insect immune repertoire: in *Aa*, the malaria vector *Anopheles gambiae* (*Ag*), and the fruit fly *Drosophila melanogaster* (*Dm*). Analysis of immune signaling pathways and response modules reveals both conservative and rapidly evolving features associated with different functional gene categories and particular aspects of immune reactions. These dynamics reflect in part continuous readjustment between accommodation and rejection of pathogens and suggest how innate immunity may have evolved.

Repeatedly during evolution, mosquitoes and other insects have adopted hematophagy to sustain abundant progeny production. In turn, blood feeding provided a new point of entry for pathogens. To counter assaults, innate immunity has evolved to recognize and respond to numerous pathogens, in a dynamic playoff where either host or pathogen may win. Although fundamental concepts mostly derive from *Dm*, *Ag* is now an important model for studies of innate immunity. A previous comparative analysis of *Ag* and *Dm* immune-related gene families (*1*) highlighted their diversification and pointed toward an expanded conceptual framework of insect innate immunity. The sequencing of the *Aa* genome (*2*) permitted deeper understanding of insect immune systems, as displayed by two quite different mosquito species that diverged ~150 million years ago (Ma) and *Dm*, which separated from them ~250 Ma. This three-way comparison is considerably more powerful than the previous *Dm-Ag* study, because it allows measuring true genetic distances rather than unrooted sequence similarities. Taking advantage of the added value from multiple species comparisons, we explore the evolutionary dynamics of innate immunity in insects and how they can address both common and species-specific immune challenges.

Multiple large-scale bioinformatic methods, manual curation, and phylogenetic analyses (*3*) identified 285 *Dm*, 338 *Ag*, and 353 *Aa* genes from 31 gene families and functional groups implicated in classical innate immunity or defense functions such as apoptosis and response to oxidative stress (table S1). Additional limited analysis of nine sequenced genomes from four holometabolous insect orders, spanning 350 million years of evolution, further defined conserved family features and assisted manual gene model curation by gene family experts. The detailed core analysis (*Aa*/*Ag*/*Dm*) is presented in the supporting online material (SOM) text and in figs. S1 to S22, and the total data set is organized into a web-accessible resource (http://cegg.unige.ch/Insecta/immunodb/), offering a comparative perspective across higher insects. All but 24 previously named *Aa* genes, as well as 79 previously unnamed *Ag* genes, were named in accordance with the nomenclature scheme devised for the *Ag* genome (*1*) with the use of additional guidelines as described in the SOM; this information will be incorporated in the forthcoming manual annotations of the VectorBase resource (www.vectorbase.org).

Our conservative bioinformatic analysis of the complete genomes identified 4951 orthologous trios (1:1:1 orthologs in the three species) and 886 mosquito-specific orthologous pairs (absent from both *Dm* and the honeybee, *Apis mellifera*). Combined bioinformatic analysis and manual curation of the immune repertoire identified 91 trios and 57 pairs, plus a combined total of 589 paralogous genes in the three species. Paralogs derive from family expansions and gene losses, or cases of exceptionally high sequence divergence obscuring phylogenetic relationships. Orthologs most likely serve corresponding functions in respective organisms, whereas paralogs may have acquired different functions.

By definition, orthologous trios represent a numerically conserved subset of genes. Nevertheless, a plot of *Dm-Aa* and *Dm-Ag* phylogenetic distances, measured in terms of amino acid substitutions, revealed that, on average, immunity trio orthologs are significantly more divergent (~20%) than the totality of trios in the genomes

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(Fig. 1A). Indeed, the immune repertoire is one of the most divergent functional groups as defined by Gene Ontology classifications (fig. S1A). Furthermore, with *Dm* as reference, several *Ag* immunity genes are considerably more divergent than their *Aa* orthologs. A similar trend among all 1:1:1 orthologs was detected, implying greater accumulation of amino acid substitutions in *Anopheles*. One hypothesis that merits detailed testing is whether this reflects a higher speciation rate and diverse habitat colonization by *Anopheles* as opposed to the more cosmopolitan *Aedes*.

Large variation exists in different immune families in their proportions of orthologous trios, mosquito pairs, and species-specific genes (Fig. 1B). Some families display exclusively species-specific genes, some mostly trios, and others intermediate variation. At one extreme are apoptosis inhibitors (IAPs), oxidative defense enzymes [superoxide dismutases (SODs), glutathione peroxidases (GPXs), thioredoxin peroxidases (TPXs), and heme-containing peroxidases (HPXs)], and class A and B scavenger receptors (SCRs), all of which show predominantly trio orthologs. At the opposite extreme are highly diverse immune effector gene families, including three shared antimicrobial peptide (AMP) families that collectively exhibit no orthologous trio and only one confident mosquito orthologous pair. The C-type lectins (CTLs), which have been implicated in immunity as opsonins and modulators of melanization (see below), are intermediate, exhibiting large expansions while retaining nine trios and one pair. The present study reaffirms the family diversity observed in our previous *Dm-Ag* comparison and further reveals substantial diversity between the two mosquito species, at just over half the evolutionary distance.

A fascinating picture emerged when we disarticulated the immune responses into sequential phases (Figs. 2 and 3). Immune responses begin with molecular recognition of microbial patterns, producing immune signals. Some signals are modulated and/or transduced before activating effector mechanisms. We observed that each of the phases is characterized by different evolutionary dynamics, which may collectively account for the flexibility of the innate immune system that enables adaptation to new challenges.

The immune recognition phase seems to achieve flexibility through divergent evolution: Gene duplications result in species- or lineage-specific expansions and generation of novel genes, whereas domain duplications lead to new gene architectures. Consequently, fruit fly and mosquito recognition proteins mostly form distinct clades within each family (see SOM). Nevertheless, sequence divergence between reduplicated recognition genes or domains remains limited, possibly reflecting the relatively limited diversity of microbial molecular patterns that are known to trigger immune responses. The peptidoglycan recognition proteins (PGRPs) and the Gram-negative binding proteins (GNBPs) are recognition receptor families that trigger signaling through Toll or Imd pathways as indicated in Fig. 2 (*4*). The Gram-negative recognition protein *Dm* PGRP-LC, which functions in the Imd pathway, and its *Anopheles* ortholog each have three functional PGRP domains; however, these are more similar within species than between species, indicating phylogenetically separate domain reduplications. A sequence gap obscures the full structure of the *Aedes PGRP-LC* ortholog, which apparently derives from the same domain reduplication events that created *Ag PGRP-LC*. Separate reduplication of two adjacent *PGRP-LC* domains in *Drosophila* generated a novel gene, *PGRP-LF*, which is absent from mosquitoes.

The function of PGRP-LC in *Dm* is antagonized by catalytic PGRPs that cleave and inactivate peptidoglycan (*5*, *6*). Mosquitoes also possess catalytic PGRPs, but most have emerged as species-specific paralogs (*Ag* PGRPS2/3 and *Aa* PGRPS4/5). The fruit fly recognizes Gram-positive bacteria activating Toll using the species-specific *Dm* PGRP-SD, as well as *Dm* PGRP-SA, which belongs to a trio and functions in conjunction with GNBP1, a recognition protein that processes polymeric peptidoglycan (*7*). The two additional *Dm* GNBPs are also fruit fly–specific; one of them, GNBP3, recognizes fungi, possibly through binding β1,3-glucans (*8*). A large expansion has generated five mosquito-specific B-type

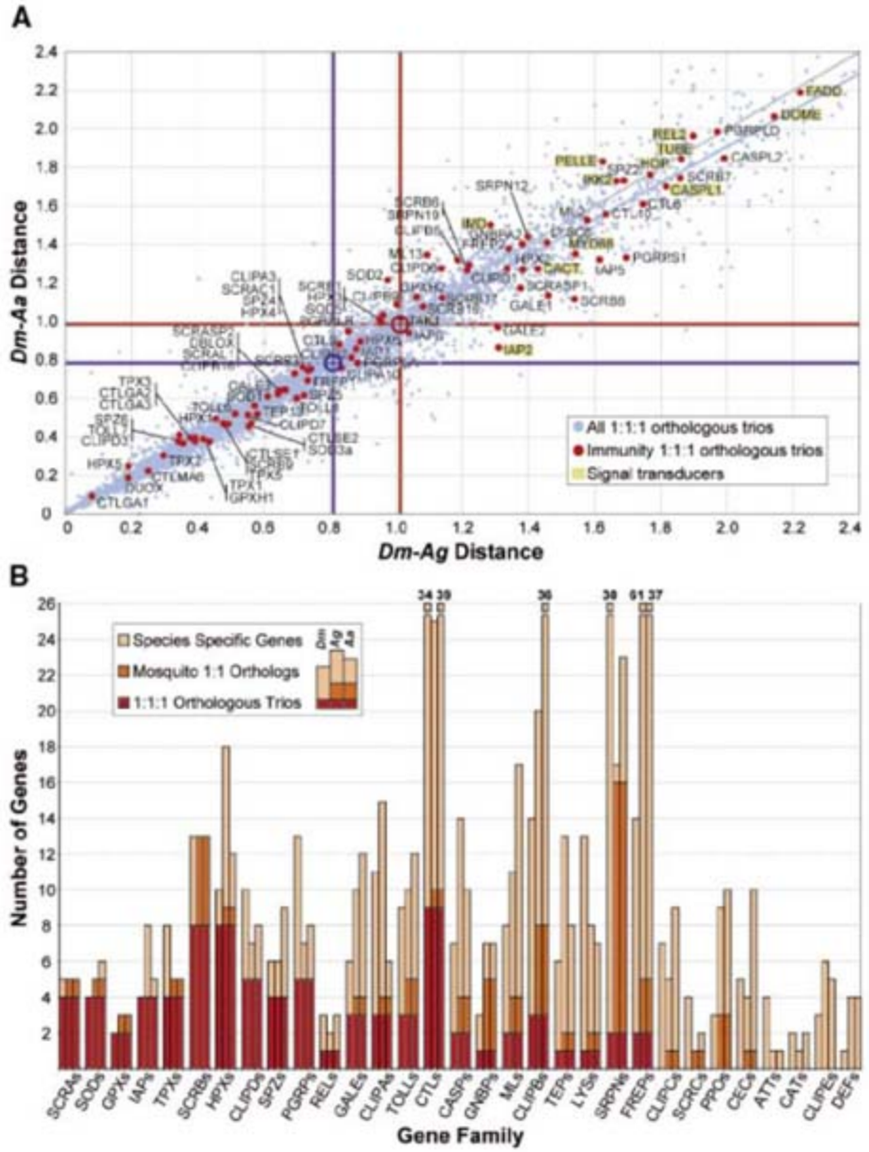
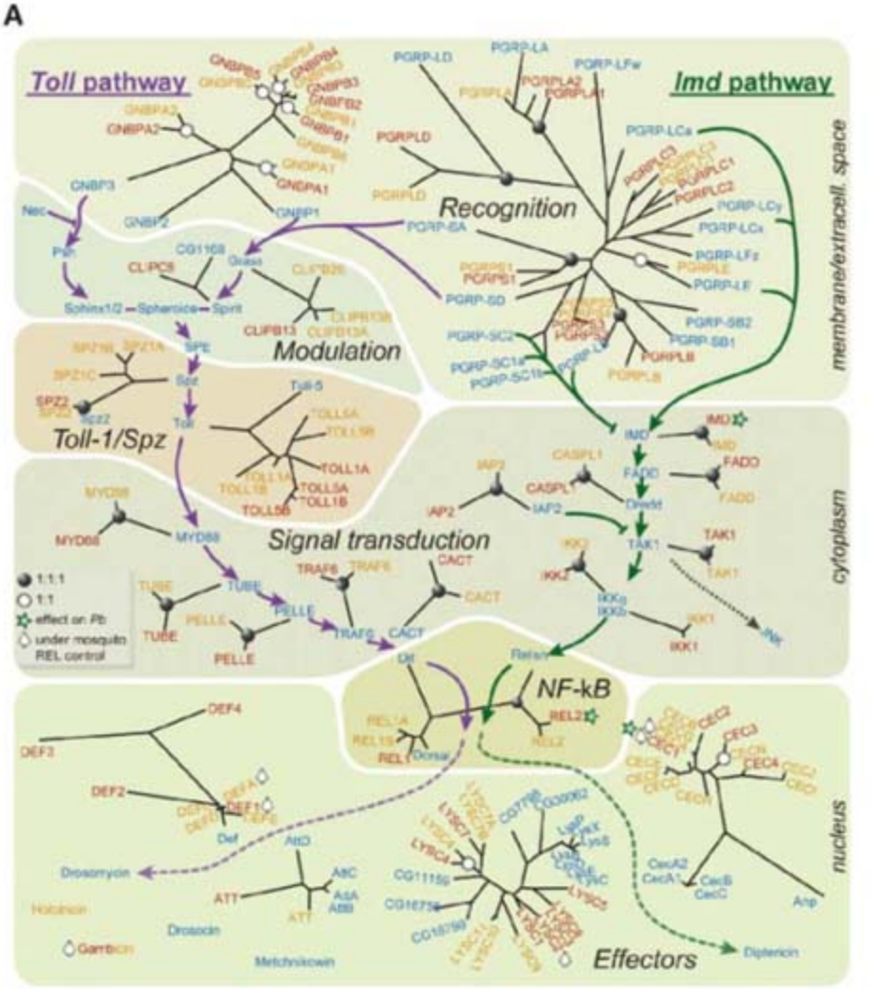


Fig. 1. (**A**) Divergence of orthologous trios. Immunity single-copy trios are compared with all single-copy trios in terms of genetic distances of each mosquito species (*Ag* or *Aa*) protein to the corresponding *Dm* ortholog (*3*) (fig. S1B). Signal transducers are highlighted. Red and blue lines indicate distance means for immunity (red dots) and all trios (blue dots), respectively. (**B**) The repertoire of putative immune-related gene families. The numbers of 1:1:1 orthologous trios (red), mosquito-specific 1:1 orthologs (orange), and species-specific genes (light brown) are summed to give the total number of genes identified in *Dm* (first bar), *Ag* (second bar), and *Aa* (third bar) for each gene (sub)family. Families are arranged from left to right, according to the decreasing proportion of 1:1:1 orthologous trios within the family. Family acronyms that are not defined in the text include: CASPs, caspases; CATs, catalases; FREPs, fibrinogen-related proteins; GALEs, galectins; MLs, MD2-like receptors.

GNBPs, distinct from the two A-type orthologous pairs that resemble fruit fly GNBPs.

Recent studies in *Ag* identified two types of putative malaria parasite recognition receptors belonging to distinct structural classes: thioester-containing proteins (TEPs) and leucine-rich repeat (LRR) proteins. Members of each class have been associated with the killing and disposal of parasites by lysis or melanization. The TEP family is related to the vertebrate complement factors C3/C4/C5 and pan-protease inhibitors α2-macroglobulins. *Ag* TEP1 binds to the surface of *Plasmodium berghei* and mediates parasite killing (*9*); it also binds to bacteria and promotes phagocytosis (*10*, *11*). TEPs exhibit only one orthologous trio and otherwise form two groups: one with both *Dm* and mosquito TEPs and another with only mosquito species-specific clades (the latter group includes *Ag* TEP1) (Fig. 3). The second class of putative receptors include LRR immune gene 1, the pioneer *P. berghei* LRR antagonist (*12*); others of similar function are *Anopheles Plasmodium*-responsive LRR 1 and LRR domain 7, which have been additionally implicated in resistance to *P. falciparum*, the human malaria parasite (*13*, *14*). Like TEP1, none of the three has identifiable orthologs in *Aa* or *Dm*.

Immune modulation is an important process that regulates both the immediate aftermath of recognition and subsequent effector functions and evolves in a "mix and match" mode. Examples are modulation of Toll pathway activation and the melanization reaction, respectively. In both contexts, modulation uses a vast reservoir of serine proteases and their inhibitors [serpins or serine protease inhibitors (SRPNs)] or other regulators, from which particular components are picked to constitute species-specific regulatory modules.



Immune phases	species specific	○ 1:1	● 1:1:1
Recognition	21	12	12
diversity by gene and domain duplication			
Modulation	13	0	0
distinct modules from large, highly divergent families			
Toll-1/Spz	14	0	3
distinct interacting pairs			
Signal transduction	0	0	36
strict orthology, coherent sequence diversification			
NF-kB	5	0	3
function transfer across paralogs			
Effectors	65	9	3
diversity by family invention & gene duplication			

Fig. 2. Evolution of immune signaling phases in insects. (**A**) Genes and gene families implicated in two immune signaling pathways, Toll and Imd (green and purple, respectively). The well-recognized phases of signaling, from recognition to effector production, are outlined. Genes known to be part of these pathways in *Dm* are indicated in blue, with their closest phylogenetic relatives in *Ag* in red and *Aa* in yellow (based on the analysis presented in the SOM). Single-copy orthologs (1:1:1) in all three genomes are indicated with solid circles at the branching node and mosquito 1:1 orthologs are indicated with open circles, respectively. *Ag* genes affecting survival of the malaria parasite *P. berghei* are marked with stars, and mosquito genes transcriptionally regulated by NF-κB–like mosquito REL factors are marked with diamonds; *Aa CECA* and *Aa DEFA* effectors are controlled by both REL1A and REL2 (*33*, *39*); similarly, *Ag* REL2 controls expression of immune effectors, including *CEC1/3* and *GAM* (*40*). *Dm* LYSs show little response to bacterial infection, but several are up-regulated after infection by microsporidia (*41*). The mosquito *Ag LYSC1/2* and *Aa LYSC11* (*LysA*) genes are up-regulated after bacterial challenge (*42*, *43*), and *Ag LYSC2* is controlled by REL1. We constructed radial trees using similarity distances of the conserved sequence cores computed by maximum likelihood. Branch-length scaling is preserved within, but not between, trees. (**B**) Gene families implicated in the three major immune phases (recognition, signal transduction, and effector production) are clearly different in relative sequence divergence (left panel; sum of branch lengths divided by number of members). Quantitative analysis of evolutionary divergence modes in all six phases defined in (A) is based on gene numbers: trios, mosquito pairs, and genes found in only one species (right panel). All signal transduction genes form trios but are maximally divergent in sequence. In contrast, effector families diversify not by sequence divergence but by gene duplication and creation of new families (e.g., Gambicin in mosquitoes and Diptericin, Drosocin, and others in *Dm*). This mode results in numerous species-specific effectors but very few trios, contrasting with the pattern seen in signal transduction. The species-specific modulators are selected separately in each species, from very large, divergent families such as SRPNs and CLIPs. Although the Toll and SPZ families are rich in trios, the mosquito genes most closely related to the *Dm* Toll-1/Spz interaction module are largely species-specific. Finally, the recognition phase shows an intermediate level of diversification, with species-specific genes approximately equal in number to the gene sum of trios and mosquito pairs; in this case, diversification arises by duplication of both genes and domains within genes [see (A)].

Successful triggering of the *Dm* Toll pathway after fungal and Gram-positive recognition engages a dedicated proteolytic activation cascade of serine proteases and SRPNs, of which several have been identified recently (*15*). None of these proteins exhibit mosquito orthologs, and only Spirit and Grass have recognizable paralogs (Fig. 2). The cascade culminates in cleavage of Spaetzle by the Spaetzle proteolytic enzyme (SPE), releasing a cytokine that binds to Toll. Mosquitoes have several genes encoding Spaetzle-like proteins (SPZs), but their SPE has not been recognized. Suggestively, the short and very specific SPE cleavage site (*16*) recurs in *Ag* CLIP-domain serine protease B5 (*Ag* CLIPB5) and *Aa* CLIPB38, which are otherwise phylogenetically unrelated.

Similarly, activation of prophenoloxidases (PPOs) to phenoloxidases (POs), the executors of melanization, is induced by a protease cascade (mostly CLIPBs). The cascade is positively and negatively regulated by a network of inactive protease homologs (CLIPAs), CTLs, and SRPNs (Fig. 3). This melanization module is tightly controlled, because it generates toxic byproducts including reactive oxygen species. Reverse genetic analyses have identified a large set of *Ag* regulators for melanization of *P. berghei* (*17*–*19*) or Sephadex beads (*20*, *21*): one SRPN, two CTLs, eight CLIPBs, and three CLIPAs (Fig. 3). Notably, all are members of mosquito-specific expansions, none has a definitive 1:1:1 ortholog, and only SRPN2 has a clear *Aa* ortholog. The reservoir of *Aa* proteases shows an underrepresentation of CLIPAs and massive expansions of CLIPBs as compared with both *Ag* and *Dm*. Finally, the melanization module may encompass additional regulators, because the genetic background determines which components are important in specific *Ag* strains (*19*).

The observed diversity of modulation components suggests that related but distinct regulatory modules may evolve in different species and even in subspecific taxa. Recruitment of individual members from very large multigene families may be followed by modulatory fine-tuning through selection imposed by particular microbes. For example, several of the genes that negatively control *P. berghei* melanization in *Ag* [*CTL4*, *CTL mannose-binding 2* (*CTLMA2*), and *SRPN2*] do not affect *P. falciparum* (*22*, *23*). Because *Ag* is a natural vector of *P. falciparum* but not of *P. berghei*, it is appealing to speculate that the sets of regulators of the melanization module evolve with and are manipulated by parasites. This modular mix and match evolution hinders detailed knowledge transfer between vector species but reinforces its importance in shaping the immune response. Future experimental studies of the melanization module in *Aa*, which can melanize bacteria and filarial worms, as well as sporozoites of the avian parasite *P. gallinaceum* (*24*, *25*), will be fruitful in further exploring this fascinating mode of immune evolution.

Although Toll-like receptors (TLRs) are found throughout the animal kingdom, phylogenetic and functional studies have suggested that insect Tolls and mammalian TLRs evolved independently (*26*). Most *Dm* Tolls serve developmental functions, and the recruitment of the Toll (Toll-1) receptor to immune signaling has been ascribed to convergent evolution. Even within insects, our analysis detects diversity: species-specific Toll expansions and only three trios. *Dm* Toll-1 has no clear orthologs; reduplications have created a clade of four *Ag* and four *Aa* genes, all related to both *Dm* Toll-1 and *Dm* Toll-5 (Fig. 2). In addition to its role in antifungal and antibacterial responses, *Dm* Toll-1 has been implicated in cellular antiviral responses (*27*). Thus, the possibility that the expanded Toll-1/Toll-5 clade in mosquitoes is related to their interactions with viruses merits detailed functional investigation. An unexpected evolutionary pattern was also observed for Spaetzle, the cytokine partner of *Dm* Toll-1, which shows three *Aa* paralogs and no identifiable *Ag* ortholog. *Aa* SPZ1C acts together with *Aa* TOLL5A to activate antifungal responses (*28*); however, the absence of an *Ag* Spaetzle ortholog raises questions about the evolution of this pair of molecules as an immune module, especially because the cytokine-Toll interaction is not required for mammalian TLR signaling. The only insect Tolls that cluster with TLRs are *Dm* Toll-9, *Ag* TOLL9, and *Aa* TOLL9A/9B. Because *Dm* Toll-9 is the only other Toll linked to *Drosophila* immunity (*29*), it is possible that this clade represents the most ancient immune-related insect Tolls. Whether these receptors can directly recognize microbial or viral immune inducers remains to be seen; it is worth noting that they are

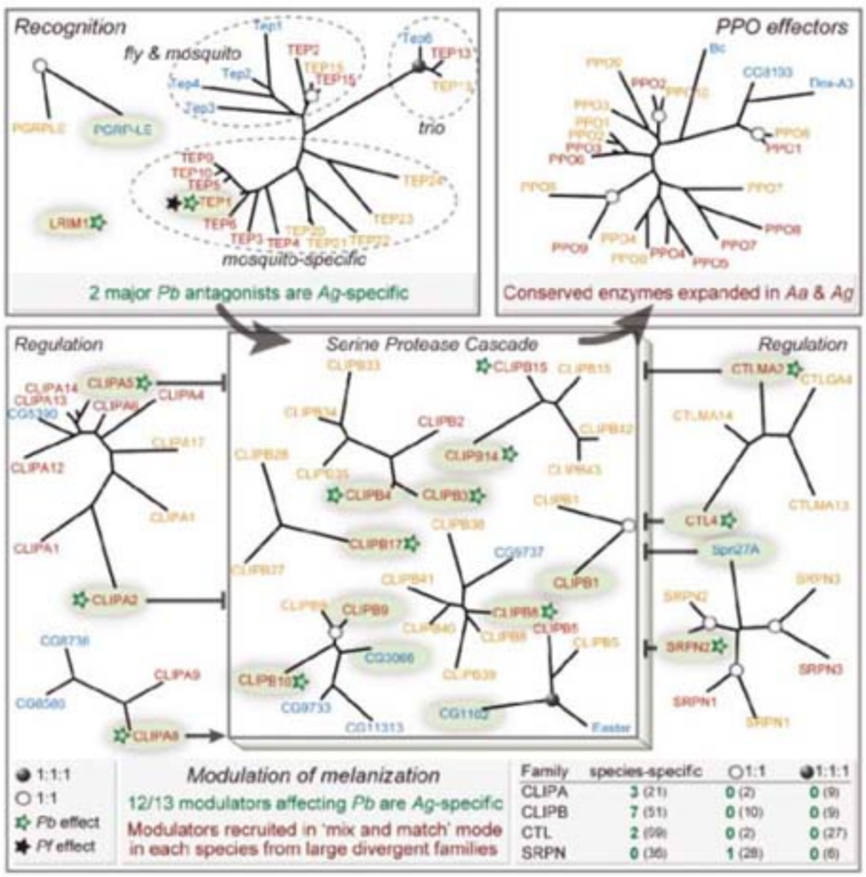


Fig. 3. The melanization immune response evolves by convergence and is based on pathogen-related, species-specific regulatory modules. Components are highlighted and shown in relation to their closest phylogenetic relatives in *Dm* (blue), *Ag* (red), and *Aa* (yellow). They are grouped in three phases: recognition, signal modulation, and effectors. TEPs exhibit only one orthologous trio and otherwise form two groups: one with both *Dm* and mosquito genes and another with species-specific mosquito clades. Recognition genes affecting *P. berghei* (*Pb*) melanization (green stars) are *Ag*-specific. Similarly, among modulators, those affecting *Pb* melanization (numbers in green in the bottom right box) are almost exclusively specific for *Ag* and are recruited from large divergent families (numbers in parentheses). In the modulation phase, CLIPB cascades are regulated positively and/or negatively by serine protease homologs (CLIPAs), CTLs, and SRPNs. Among those, CLIPB1, 4, 8, 9, and 10 are involved in melanization of Sephadex beads. The PPO effectors remain conserved in sequence to preserve their enzymatic function, but the family is expanded in mosquitoes. *Ag* genes marked with black stars affect survival of *P. falciparum* (*Pf*). Single-copy orthologs (1:1:1) in all three genomes are indicated with solid circles, and mosquito 1:1 orthologs are indicated with open circles on respective nodes. We constructed radial trees using similarity distances of the conserved sequence cores computed by maximum likelihood, with branch-length scaling preserved within but not between trees.

more similar to lipid-binding TLRs rather than to nucleic acid–binding TLRs.

Signal transduction components exhibit an unexpected mode of evolution. Rather than duplicating to create novel cascades responding to distinct challenges, or picking up members of multiprotein families to promote adaptive interactions, these components show robustness, maintaining their distinctive identity and functionality in the face of sequence evolution. The cytoplasmic signal transduction of the Toll pathway includes a chain of interacting partners, almost invariably encoded by orthologous trios: myeloid differentiation factor 88 (MYD88), TUBE, PELLE, tumor necrosis factor receptor–associated factor 6 (TRAF6), and CACT (Fig. 2). The same is true for the components of the IMD pathway: IMD, Fas-associated death domain protein (FADD), Dredd (CASPL1), IAP2, transforming growth factor β–activated kinase (TAK1), and inhibitor of nuclear factor κB kinase subunits γ and β (IKKγ and IKKβ). Despite persistent orthology, these components show marked divergence in sequence (Fig. 1A). A similar pattern is observed in the signal transducers Dome and Hop of the immune signaling Janus kinase–signal transducers and activators of transcription (JAK-STAT) pathway, which is activated in *Dm* by virus infections (*30*). We hypothesize that the requirement for these factors to interact productively with others in the same chain causes escalating sequence divergence: A mutation in one may enhance the acceptability of certain mutations in its interacting partner, maintaining pathway function through coherent evolution rather than stasis. Consistent with this interpretation, evidence has been reported for an association between natural sequence variation of core signaling pathway components and immune competence in *Drosophila* (*31*). Similar evolutionary patterns are detected among members of the RNA interference antiviral pathway, Dicer-2 and Ago-2 (*32*), which also form highly divergent trios.

Signal transduction culminates in the next phase: nuclear translocation of transcription factors. The cytoplasmic nuclear factor κB (NF-κB) transcription factors remain inactive until a processed immune signal frees them from inhibitors, permitting their entry into the nucleus and transcription of effector genes. The evolutionary pattern in this phase combines aspects observed in other phases. The NF-κBs of the Imd pathway [Relish in *Dm* and Rel-like NF-κB protein 2 (REL2) in mosquitoes] form an orthologous trio that displays high sequence divergence, as in signal transducer trios (Figs. 1A and 2). A recent duplication in *Aa* has resulted in an orthologous quartet (*Ag* REL1, *Dm* Dorsal, *Aa* REL1A, and *Aa* REL1B). In contrast, Dif is absent from both mosquito species, although the intronless *Aa REL1B* gene may have originated by retrotransposition. Transgenic analysis has shown that REL1A controls *Aedes* antifungal responses, as does Dif in *Dm* (*33*); this represents an interesting case of functional transfer between paralogs. STAT, the transcription factor of the JAK-STAT pathway, shows high sequence divergence like REL2 and has been duplicated in *Ag*.

Immune effectors are required to target and neutralize the microbial source of the immune signal. We observed varied evolutionary dynamics for different categories of effectors, reflecting their modes of action. Those acting directly on microbes diversify rapidly or are species-specific, whereas effector enzymes that produce chemical cues to attack invaders remain conserved but independently expand in each species.

The production of AMPs, which act on bacterial membranes causing lysis, is a classic immune-inducible effector response (Fig. 2). Seven AMP families exist in *Dm*, but only three of them were detected in mosquitoes: Defensins (DEFs), cecropins (CECs), and attacins (ATTs) are highly diverse, together displaying no orthologous trio and only one confident 1:1 orthologous pair. Conversely, gambicins are only encountered in mosquitoes. The apparent paucity of mosquito AMPs in contrast to *Dm* may be attributable to different prevalence of bacteria in their respective environments.

As diverse as AMPs, the large family of antibacterial peptidoglycan-hydrolyzing lysozymes (LYSs) shows only one identifiable trio and one mosquito pair among 28 members (Fig. 2). A marked expansion in *Dm* is ascribable to the use of LYSs for digestion of bacteria as a food resource: These peptides are atypically acidic and are expressed in the midgut but not in other immune tissues (*34*). Apart from these digestive *Dm* LYSs, the family forms two groups: one with both *Dm* and mosquito LYSs and the other with only species-specific clades of mosquito LYSs—a very similar pattern to that observed for TEPs, which are also thought to function both as recognition receptors and as complement effectors.

The family of PPO melanization effectors has expanded greatly in mosquitoes as compared with *Dm* and larger model insects. *Ag* PPO1/*Aa* PPO6 is the only orthologous pair that clusters with *Dm* PPOs; the remaining 17 mosquito PPOs form a distinct clade, created by reduplication events both before and since *Ag-Aa* diverged (Fig. 3). The invariable catalytic activity of POs (conversion of tyrosine to melanin) is likely to restrict their functional diversification, suggesting that observed expansions may reflect diversification to accommodate differential developmental, topological, or temporal activation. Indeed, several *Aa* and *Ag* PPOs show developmental or physiological specificity (*35*, *36*).

In *Ag*, increased systemic levels of hydrogen peroxide (H_2O_2) have been associated with *Plasmodium* melanization (*37*). H_2O_2 is used as an electron acceptor by HPXs that catalyze various oxidative reactions. This effector family shows a small expansion in *Aa* and a large one in *Ag*, while retaining a set of eight orthologous trios including *DUOX* (dual HPX and NADPH-oxidase, where NADPH is the reduced form of nicotinamide adenine dinucleotide phosphate). The latter is associated with peroxidase-mediated nitration during the apoptotic response of midgut cells to *Plasmodium* invasion (*38*). Numerous trio orthologs of HPXs and other enzyme families implicated in oxidative defense show low sequence divergence, suggestive of constraints to preserve ubiquitous catalytic activities.

The availability of the genome sequences of distantly related insects has allowed us to apply comparative genomic methods to analyze the evolutionary dynamics of the insect innate immune repertoires. Notably, we identified distinct and seemingly contrasting evolutionary modes characterizing different immune modules, which together serve to provide a flexible system capable of adapting to new challenges. The repertoire of recognition receptors of microbial groups such as bacteria and fungi, which are encountered by all species, is achieved through expansion and fine-tuning of model genes. New functions (e.g., recognition of malaria parasites) are acquired from genes bearing powerful and ancient recognition domains such as LRRs. Protein networks modulating immune signals are assembled independently in each species, in the mix and match mode of evolution described as "bricolage" by François Jacob; they therefore coevolve with pathogens and may be subject to evasion. Pathways of signal transduction, on the other hand, remain highly conserved, and their constituent genes seem to evolve always in concert. Finally, effector mechanisms follow evolutionary patterns that depend on their mode of action; most are highly divergent or even species-specific, in contrast to the ancient, conserved oxidative defense mechanisms.

Recognition of the role of Toll in *Drosophila* immunity led directly to the identification of TLRs as a fundamental aspect of mammalian innate immunity. Similarly, the diverse evolutionary modes of insect immunity that we detected in the present study can guide future studies on the evolution of innate immune mechanisms in vertebrates and other animals. They can also facilitate targeted studies of immunity in the two mosquito species, which together transmit some of the most devastating infectious diseases of humankind.

References and Notes

1. G. K. Christophides *et al.*, *Science* **298**, 159 (2002).
2. V. Nene *et al.*, *Science* **316**, 1718 (2007); published online 17 May 2007 (10.1126/science.1138878).
3. Materials and methods are available as supporting material on *Science* Online.
4. L. Wang, P. Ligoxygakis, *Immunobiology* **211**, 251 (2006).
5. A. Zaidman-Remy *et al.*, *Immunity* **24**, 463 (2006).
6. V. Bischoff *et al.*, *PLoS Pathog.* **2**, e14 (2006).
7. L. Wang *et al.*, *EMBO J.* **25**, 5005 (2006).
8. M. Gottar *et al.*, *Cell* **127**, 1425 (2006).
9. S. Blandin, E. A. Levashina, *Mol. Immunol.* **40**, 903 (2004).
10. E. A. Levashina *et al.*, *Cell* **104**, 709 (2001).
11. L. F. Moita *et al.*, *Immunity* **23**, 65 (2005).
12. M. A. Osta, G. K. Christophides, F. C. Kafatos, *Science* **303**, 2030 (2004).
13. M. M. Riehle *et al.*, *Science* **312**, 577 (2006).

14. Y. Dong *et al.*, *PLoS Pathog.* **2**, e52 (2006).
15. Z. Kambris *et al.*, *Curr. Biol.* **16**, 808 (2006).
16. I. H. Jang *et al.*, *Dev. Cell* **10**, 45 (2006).
17. J. Volz *et al.*, *J. Biol. Chem.* **280**, 40161 (2005).
18. K. Michel *et al.*, *EMBO Rep.* **6**, 891 (2005).
19. J. Volz *et al.*, *Cell. Microbiol.* **8**, 1392 (2006).
20. E. Warr *et al.*, *Insect Biochem. Mol. Biol.* **36**, 769 (2006).
21. S. M. Paskewitz, O. Andreev, L. Shi, *Insect Biochem. Mol. Biol.* **36**, 701 (2006).
22. A. Cohuet *et al.*, *EMBO Rep.* **7**, 1285 (2006).
23. K. Michel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16858 (2006).
24. J. F. Hillyer, S. L. Schmidt, B. M. Christensen, *J. Parasitol.* **89**, 62 (2003).
25. B. M. Christensen *et al.*, *Trends Parasitol.* **21**, 192 (2005).
26. J. L. Imler, L. Zheng, *J. Leukocyte Biol.* **75**, 18 (2004).
27. R. A. Zambon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7257 (2005).
28. S. W. Shin, G. Bian, A. S. Raikhel, *J. Biol. Chem.* **281**, 39388 (2006).
29. J. Y. Ooi *et al.*, *EMBO Rep.* **3**, 82 (2002).
30. C. Dostert *et al.*, *Nat. Immunol.* **6**, 946 (2005).
31. B. P. Lazzaro, B. K. Sceurman, A. G. Clark, *Science* **303**, 1873 (2004).
32. R. P. van Rij *et al.*, *Genes Dev.* **20**, 2985 (2006).
33. G. Bian *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13568 (2005).
34. S. Daffre *et al.*, *Mol. Gen. Genet.* **242**, 152 (1994).
35. H. M. Muller *et al.*, *J. Biol. Chem.* **274**, 11727 (1999).
36. J. S. Li *et al.*, *Insect Biochem. Mol. Biol.* **35**, 1269 (2005).
37. S. Kumar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14139 (2003).
38. S. Kumar *et al.*, *J. Biol. Chem.* **279**, 53475 (2004).
39. S. W. Shin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 2616 (2003).
40. S. Meister *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11420 (2005).
41. K. Roxstrom-Lindquist, O. Terenius, I. Faye, *EMBO Rep.* **5**, 207 (2004).
42. R. J. Ursic Bedoya *et al.*, *Insect Mol. Biol.* **14**, 89 (2005).
43. B. Li *et al.*, *Gene* **360**, 131 (2005).
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Supporting Online Material

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Culling Prey Promotes Predator Recovery—Alternative States in a Whole-Lake Experiment

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Many top-predator fish stocks in both freshwater and marine systems have collapsed as a result of overharvesting. Consequently, some of these communities have shifted into seemingly irreversible new states. We showed, for predators feeding on prey that exhibit food-dependent growth, that culling of fish prey may promote predator recovery. We removed old stunted individuals of a prey-fish species in a large, low-productive lake, which caused an increase in the availability of small-sized prey and allowed the predator to recover. The shift in community state has been sustained for more than 15 years after the cull ended and represents an experimental demonstration of an alternative stable state in a large-scale field system. Because most animals exhibit food-dependent growth, shifts into alternative stable states resulting from overcompensating prey growth may be common in nature and may require counterintuitive management strategies.

Rapid changes observed in many ecological systems, such as the collapse of major fish stocks (*1*–*4*), have prompted an increased interest in alternative stable states during recent years (*5*, *6*). Theoretical studies (*7*, *8*) suggest that size-selective predation (*9*–*11*) may be a major mechanism behind shifts between alternative stable states if reduced competition for resources among remaining prey (*6*, *12*) accelerates prey growth. Predation on small individuals in this case leads to an overcompensating response because surviving prey mature more rapidly and achieve higher population reproductive outputs. Counterintuitively, densities of small prey hence increase and not decrease when predators forage on such small prey individuals (*7*, *13*). Thus, size-selective predators shape the biotic environment to their own advantage. These predator-prey systems are, however, prone to irreversible collapse of the predator if overharvested. A drop in predator density causes prey to grow and reproduce more slowly and consequently produce lower abundances of vulnerable, small-sized prey. This change in prey-size distribution subsequently prohibits recovery of the predator, making the collapse seemingly irreversible (*7*, *8*). Next to the predator-prey state the community thus possesses an alternative stable state with only prey (*7*). Size-selective harvesting of prey may offer a route to predator recovery, because it should stimulate rates of prey growth and reproduction and thereby shift prey-size distribution toward smaller individuals. Once the prey-size constraint for recovery is lifted, the recovered predator population should itself be able to sustain the system in the new state.

In the early 1900s, the top-predator brown trout (*Salmo trutta*) was the only species in the low-productive Lake Takvatn, in northern Norway (*14*–*16*). Overharvesting reduced trout to low levels, and Arctic charr (*Salvelinus alpinus*)—prey for, but also a potential competitor of, small brown trout for invertebrates (*14*, *16*–*19*)—was introduced in about 1930. The charr soon dominated the fish community, and by 1980 trout were almost absent (Figs. 1 and 2, A and D). To improve lake fisheries, 666,000 charr (31.3 metric tons) were removed during 1984 to 1989 (*14*, *15*). By 1991 charr density had decreased by 80%, subsequently rebounded to less than half its 1984 density, and ultimately exhibited a decelerating decrease toward a new steady state (1992 to 2006, regression $F_{1,14} = 14.7$, $P = 0.002$) (Fig. 1). Trout density increased from 1989 to 1992, remaining steady afterward (1992 to 2006, regression $F_{1,14} = 0$, $P = 0.99$). On average (1992 to 2006), the trout density was 12% of the charr density, similar to the value of 15% observed in a control lake (*16*). Although charr may compete with small trout for food (*14*, *17*, *18*), we found no evidence for a negative density–dependent effect of either total charr density or density of charr <150 mm in size on the body condition of 100-mm trout (regressions, $F_{1,16} = 0.10$ to 2.27, $P > 0.1$).

We generated seven testable expectations of this prey-culling management strategy with an existing resource-prey-predator, food-chain model (*7*, *13*), in which prey exhibit food-dependent growth and reproduction and predators forage on small prey only. At low densities, invading predators cannot increase in density in the stable prey-resource state (Fig. 3). Culling prey induces oscillations in prey density and strong pulses in prey recruitment, which allow predators to increase in numbers and reach high densities. Predators can subsequently control the prey, driving the system toward an alternative, stable resource-prey-predator equilibrium (Fig. 3) characterized by (1) lower prey density (smallest-size

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classes excluded), (2) higher predator density, (3) broader prey-size distribution, (4) higher individual prey growth rate, (5) higher density of small-size classes of prey, and (6) improved body condition of predators (*7*, *13*). Finally, the route to the new equilibrium state occurs via harvesting-induced oscillations (*7*) (Fig. 3).

We compared these seven theoretical expectations with data from the whole-lake manipulation in Takvatn. First and second, the density of charr (young-of-the-year charr excluded) decreased and that of trout increased (predictions 1 and 2) (Fig. 1). After the perturbation, both populations approached new equilibrium densities sustained for more than 15 years (more than two generations), which is long enough to conclude that the new state represents an alternative stable state (*20*, *21*). In addition, we found no relationships between the shift in the fish community and lake transparency or temperature, arguing against such environmental factors being responsible for the observed shifts (regressions, $F_{1,17} = 0.00$ to 0.19, $P > 0.1$). Moreover, thinning experiments with allopatric charr populations showed return times to the initial population structure within 5 to 8 years (*22*), i.e., in less than half the post-thinning period in Takvatn. Third, the charr size distribution changed dramatically (prediction 3): Before manipulation, the charr population was densely packed with a high dominance of individuals 161 to 211 mm in size (1980 and 1981, Simpson's measure of dominance = 0.13 and 0.23, respectively) (Fig. 2A), which changed to a markedly reduced dominance of a few size classes (Simpson's measure of dominance: mean 0.05, range 0.02 to 0.08; comparison postperturbation years, 1990 to 2006, versus pre-perturbation years, 1980 to 1981, Mann-Whitney U test, two-tailed, $P = 0.022$) (Fig. 2, B and C). Fourth, compared with pre-perturbation years, the growth of individual charr increased substantially in all postperturbation years (prediction 4) (two-way analyses of variance, year class born in 1976 versus those born in 1984, 1989, 1994, and 1999, respectively, $F_{2,5} = 85.9$ to 245.5, $P < 0.001$) (Fig. 2D). It should be stressed that the growth curve for pre-perturbation years with its monotonic decrease with age and very narrow standard errors showed that the growth of Arctic charr was consistently low for the entire pre-perturbation period 1980 to 1984. In contrast, growth of individual Arctic charr after perturbation showed temporal variation depending on charr density (*15*), although it was consistently higher than in pre-perturbation years (Fig. 2D). In contrast to the charr population in Takvatn, no changes in either size distribution or growth rate of charr were observed in the nearby control lake during 1979 to 1999, and both growth rates and size distributions (Simpson's measure of dominance = 0.06) were similar to that of post-thinning charr in Takvatn (fig. S1).

As a critical prediction for establishing the mechanism causing the alternative stable states, the availability of small-sized charr preyed upon by trout should increase (prediction 5). Indeed, on average, the charr available for a 400-mm trout [charr size range 4 to 160 mm, optimal size 80 mm (*17*)] almost doubled in size (Fig. 4A), with even larger increases for smaller trout (300-mm trout, 3.2 times; 350-mm trout, 2.7 times). Compared with pre-perturbation years (1980 to 1981), estimated trout encounter rates (Fig. 4A) with charr were higher in all but three post-perturbation years (1990 to 2006, except 1990, 1996, and 1998). Also consistent with expectations, we found harvesting-induced oscillations in the charr population (prediction 7) reflected in a very distinct periodicity in charr availability for brown trout (1990 to 2006, peaks: autocorrelation $lag_4 = -0.69$, $P = 0.017$; dips: autocorrelation $lag_8 = 0.45$, $P = 0.008$; peaks: partial autocorrelation $lag_4 = -0.69$) (Fig. 4B) and resulting from oscillations in charr recruitment [capture per unit effort (CPUE) of charr <150 mm, 1990 to 2006, peaks: autocorrelation $lag_8 = -0.40$, $P = 0.024$; valleys: autocorrelation $lag_4 = 0.66$, $P = 0.02$; valleys: partial autocorrelation $lag_4 = -0.568$] (Fig. 4, C and D). The abundance of charr <150 mm, including in the perturbation years, also clearly suggests that the system approaches an equilibrium through damped oscillations (Fig. 4C). Finally, the cyclic availability of charr after the perturbation allows us to test whether increased availability of charr results in

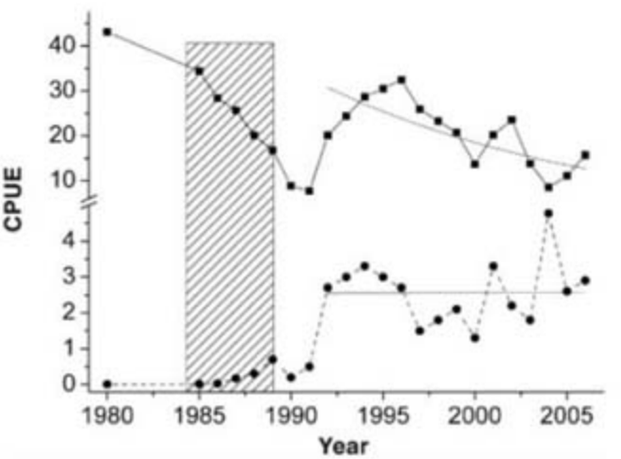

Fig. 1. Changes in abundance of Arctic charr (squares, solid lines) and brown trout (circles, dashed lines) in Lake Takvatn during 1984 to 2006. Trend lines are inserted for the period 1992 to 2006. Hatched area shows period of charr culling.

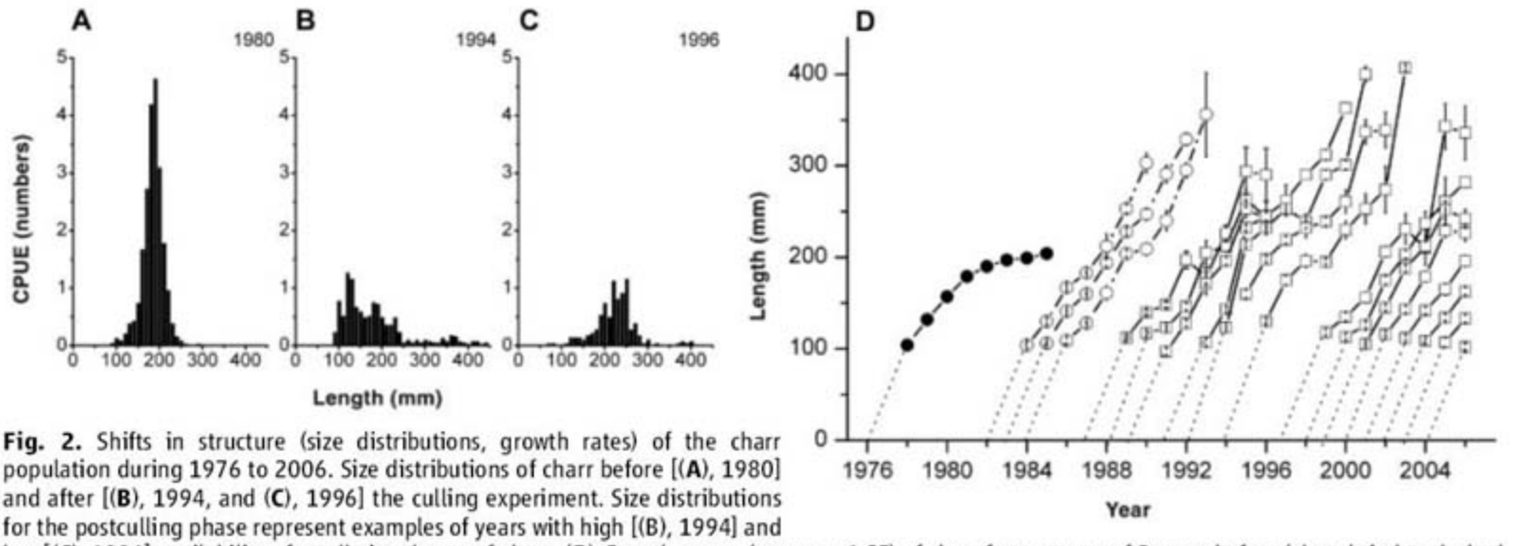

Fig. 2. Shifts in structure (size distributions, growth rates) of the charr population during 1976 to 2006. Size distributions of charr before [(**A**), 1980] and after [(**B**), 1994, and (**C**), 1996] the culling experiment. Size distributions for the postculling phase represent examples of years with high [(B), 1994] and low [(C), 1996] availability of small-size classes of charr. (**D**) Growth curves (means ± 1 SE) of charr from an age of 2 years before (closed circles, dashed line), during (open circles, dashed-dotted lines), and after (squares, solid lines) the perturbation of charr. Data from pre-perturbation period (year-class 1976) are based on samples from 1980 and 1981.

increased trout performance (prediction 6). In support of expectations, we found a positive relationship between charr availability and the body condition of trout in the same year (regression, $r^2 = 0.46$, $F_{1,16} = 12.5$, $P = 0.03$) (fig. S2). Furthermore, fluctuations in charr availability covaried with total trout abundance 2 years later (cross-correlation, $lag_2 = 0.59$), providing further support for the conclusion that competition between charr and trout was negligible.

Our experimental demonstration of alternative stable states in a large lake contrasts markedly in scale with previous experiments restricted to laboratory or field enclosure systems (*21*, *23*, *24*). The results show that differences in charr size distribution induced by food-dependent growth were pivotal for creating an alternative stable state. Because the overwhelming majority of organisms exhibit food-dependent growth, including taxa such as insects, fish, reptiles, and amphibians (*25*, *26*), overcompensation resulting from food-dependent growth could be a major mechanism giving rise to alternative stable states in many ecological systems. Our results are also relevant to interactions where the average size differences between predator and prey are greater than those between brown trout and Arctic charr, because the main predatory effect may still be directed toward the smallest prey sizes (*27*), which is crucial for the alternative stable state to occur.

Many fish communities, including the Arctic charr–brown trout systems, simultaneously suffer from low levels (or indeed the complete absence) of top predators and stunting of prey. Both predator stocking and prey culling have been advanced as management tools to counteract this situation (*1*, *4*, *28*, *29*). Our modeling studies (*7*, *13*) suggest that piscivore stocking must be rather high to be successful, and hence this is not a practical management strategy in many cases. Prey culling may be a more realistic approach to improve the status of fish stocks in a sustainable way in large lakes such as Takvatn. We finally argue that the risk of irreversible collapse from overharvesting depends not only on the life history of the target species but also on the life history of its prey. Thus, changes in size distributions of prey fish may be a sensitive indicator for risk of collapse in predatory fish (*7*).

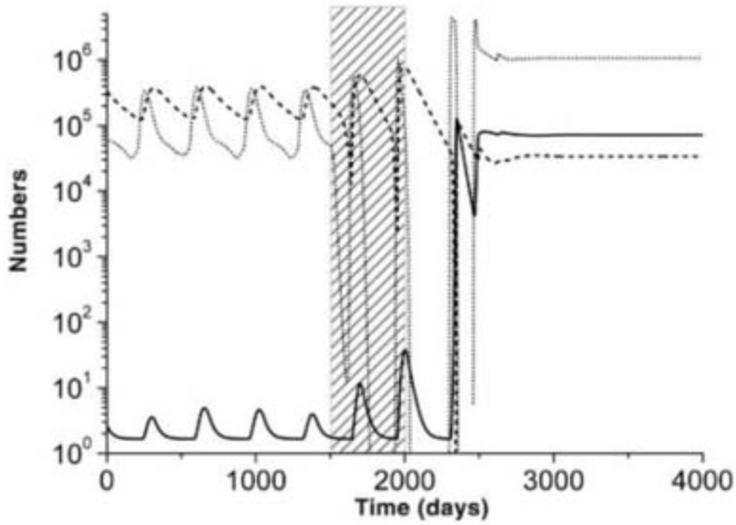

Fig. 3. Model predictions of culling size-structured prey on predator recovery. Continuous invasion of predators (solid line) until time = 1500 days is unsuccessful. At time = 1500 to 2000 days, heavy harvesting on prey (dotted line, vulnerable juveniles; dashed line, invulnerable juveniles and adults) is imposed (hatched area), leading to successful establishment of the predator. The perturbation induces oscillations in both predator and prey during a transient phase before the system reaches an alternative equilibrium.

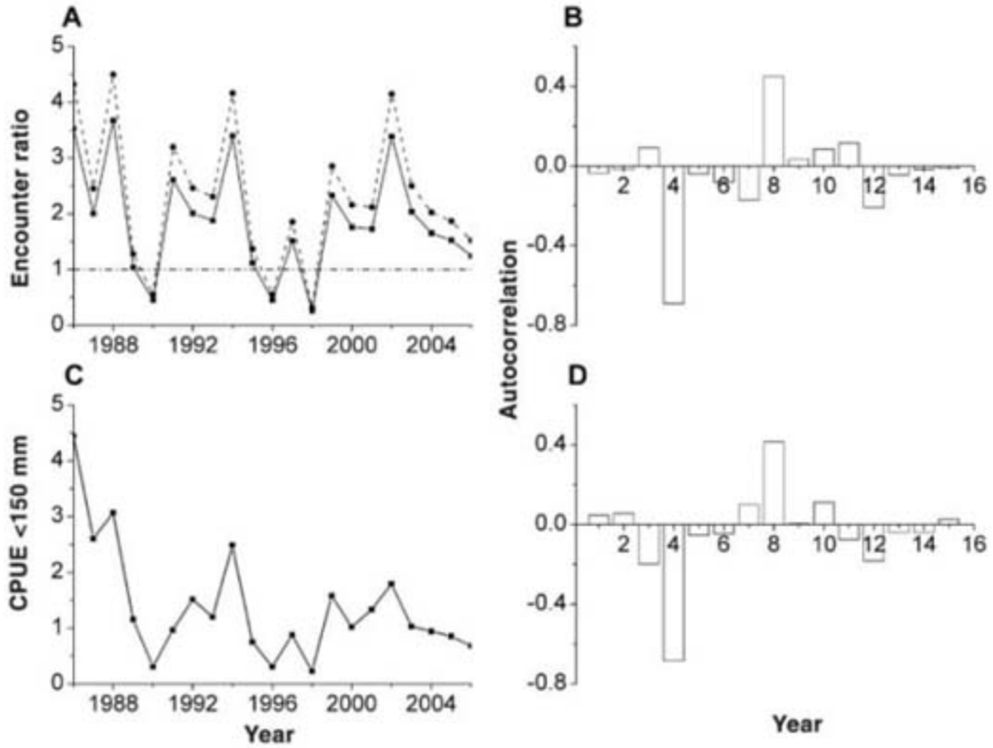

Fig. 4. Cyclic dynamics in charr availability. (**A**) Encounter rates of a 400-mm brown trout with charr in 1986 to 2006 relative to two pre-perturbations years (1980, squares and solid lines; 1981, circles and dashed lines) expressed as a ratio. (**B** and **D**) Autocorrelation in encounter rate (B) and charr <150 mm CPUE (D) for the period 1990 to 2006 showing an 8-year periodicity (negative correlations are peaks in availability, positive correlations are dips in availability). (**C**) Capture per unit effort (CPUE) of charr <150 mm.

References and Notes

1. P. A. Jansen, A. G. Finstad, A. Langeland, *Environ. Biol. Fish.* **64**, 313 (2002).
2. J. A. Hutchings, *Nature* **406**, 882 (2000).
3. J. R. Post *et al.*, *Fisheries* **27**, 6 (2002).
4. J. A. Hutchings, *Can. J. Fish. Aquat. Sci.* **62**, 824 (2005).
5. M. Scheffer, S. R. Carpenter, J. A. Foley, C. Folke, B. Walker, *Nature* **413**, 591 (2001).
6. C. Mora *et al.*, *Science* **314**, 758 (2006).
7. A. M. De Roos, L. Persson, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 12907 (2002).
8. T. van Kooten, A. M. De Roos, L. Persson, *J. Theor. Biol.* **237**, 67 (2005).
9. W. M. Tonn, C. A. Paszkowski, I. J. Holopainen, *Ecology* **73**, 951 (1992).
10. J. M. Chase, *Am. Nat.* **154**, 559 (1999).
11. R. H. Paine, J. C. Castillo, J. Cancino, *Am. Nat.* **125**, 679 (1985).
12. L. Persson, J. Andersson, E. Wahlström, P. Eklöv, *Ecology* **77**, 900 (1996).
13. A. M. De Roos, L. Persson, E. McCauley, *Ecol. Lett.* **6**, 473 (2003).
14. A. Klemetsen *et al.*, *Environ. Biol. Fish.* **64**, 39 (2002).
15. P.-A. Amundsen, R. Knudsen A. Klemetsen, *J. Anim. Ecol.* **76**, 149 (2007).
16. Materials and methods and information about interactions between charr and brown trout are available on *Science* Online.
17. J. H. L'Abée-Lund, A. Langeland, H. Saegrov, *J. Fish Biol.* **41**, 91 (1992).
18. P. A. Jansen, H. Slettvold, A. G. Finstrad, A. Langeland, *Can. J. Fish. Aquat. Sci.* **59**, 6 (2002).
19. P. Hyvärinen, A. Huusko, *J. Fish Biol.* **68**, 87 (2006).
20. J. H. Connell, W. P. Sousa, *Am. Nat.* **121**, 789 (1983).
21. A. Schröder, L. Persson, A. M. De Roos, *Oikos* **110**, 3 (2005).
22. L. Johnson, *Imperfect Symmetry—Thermodynamics in Ecology and Evolution* (Torgoch, Victoria, BC, 2002).
23. J. M. Chase, *Ecol. Lett.* **6**, 733 (2003).
24. O. J. Schmitz, *Ecol. Lett.* **7**, 403 (2004).
25. K. P. Sebens, *Annu. Rev. Ecol. Syst.* **18**, 371 (1987).
26. E. E. Werner, in *Size-Structured Populations—Ecology and Evolution*, B. Ebenman, L. Persson, Eds. (Springer, Berlin, 1988), pp. 60–81.
27. D. E. Dupliesa, *ICES J. Mar. Sci.* **62**, 412 (2005).
28. C. Walters, J. F. Kitchell, *Can. J. Fish. Aquat. Sci.* **58**, 39 (2001).
29. T. W. Edison *et al.*, *N. Am. J. Fish. Manage.* **26**, 800 (2006).

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Materials and Methods

Figs. S1 and S2

References

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Influence of Phylogeny on Fungal Community Assembly and Ecosystem Functioning

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Ecology seeks to explain species coexistence and its functional consequences, but experimental tests of mechanisms that simultaneously account for both processes are difficult. We used an experimental mycorrhizal plant system to test whether functional similarity among closely related species (phylogenetic conservatism) can drive community assembly and ecosystem functioning. Communities were constructed with the same number of fungal species, but after 1 year of growth, realized species richness was highest where the starting species were more distantly related to each other. Communities with high realized species richness also stimulated plant productivity more than those with low realized species richness. Our findings suggest that phylogenetic trait conservatism can promote coexistence because of reduced competition between distinct evolutionary lineages and enhance ecosystem function because of functional complementarity among those same lineages.

Although it has long been recognized that ecological communities are not random collections of species, ecologists still seek to understand the processes that shape community assembly (*1*–*4*). One hypothesis that explains nonrandom species assemblages is that competitive interactions limit the long-term coexistence of species with similar fundamental niches (*2*, *5*–*7*). If closely related species share a fundamental niche (niche conservatism), competitive exclusion will cause communities to be made up of species that are phylogenetically overdispersed, or more distantly related to each other than would be expected by chance (*2*, *5*, *8*–*10*). This hypothesis is difficult to test directly because the spatial and temporal scales of the critical processes in plant and animal communities are typically too large for manipulation (*11*). Recent research indicates that the degree of phylogenetic dispersion varies across communities and depends on the level of phylogenetic relatedness within a particular community and the spatial scale of species interactions (*3*, *10*, *12*–*14*). However, this evidence is correlative rather than causative because most previous studies have been confined to comparative analyses of existing communities (*15*, *16*). In addition, the strength of a phylogenetic signal in the species assemblage of communities is often obscured by stochastic processes and dispersal limitations (*8*).

Using a model mycorrhizal plant community, we experimentally determined whether community assembly depends on the phylogenetic relatedness of species. The model community consisted of sympatric arbuscular mycorrhizal fungi (AMF) growing on plant roots of *Plantago lanceolata* (*17*) (Fig. 1). The arbuscular mycorrhizal symbiosis is ideal for testing hypotheses about community assembly for two reasons. First, the small size and short generation time of the organisms allow us to manipulate and observe ecologically meaningful interactions in tractable experimental units on a short time scale. Second, most described AMF are confined to three distinct taxonomic families (Glomeraceae, Acaulosporaceae, and Gigasporaceae) within two orders (Glomerales and Diversisporales) (*18*) in which functional traits associated with spatial niche requirements are phylogenetically conserved (*19*) (Fig. 2). For example, the majority of fungal biomass in the Gigasporaceae is found in the hyphae that are located outside the plant root (Fig. 2, A and B). In contrast, the majority of fungal biomass in the Glomeraceae is found in hyphae growing inside the root (Fig. 2, A and B). The Acaulosporaceae form a third distinct group, because species in this taxon produce low biomass inside and outside the root (Fig. 2, A and B).

Species from these major evolutionary lineages were sampled to form experimental communities. We manipulated the level of phylogenetic relatedness in the species pool by constructing communities sampled from all three AMF families (relatively overdispersed) or from two or fewer families (relatively underdispersed) (Fig. 1). We predicted that species within each family were less likely to coexist with each other because of similar spatial niche requirements. In contrast, we expected that taxa from distinct lineages such as the Gigasporaceae and the Glomeraceae should coexist because they each specialize on different spatial components of the rhizosphere.

We found that community assembly depended on phylogenetic relatedness. Experimental communities were constructed with eight AMF species, but after 1 year of growth, realized species richness was highest in those communities that were assembled using taxa from all three families as compared to those communities assembled using taxa from two or fewer families (Fig. 2C). Realized species richness after 1 year was >80% of the initial value in communities with representatives from all three families. In contrast, communities made up largely of species from one family retained <40% of the initial species pool. We also found that realized species richness in phylogenetically overdispersed communities was similar regardless of the identity of the sampled species within each family (Fig. 2C), a result consistent with our expectation that there is trait conservatism and therefore a degree of functional redundancy within each AMF family (Fig. 2, A and B, and table S1) (*19*).

We also tested whether the level of phylogenetic dispersion in an AMF community could be influenced by abiotic factors (*1*). If the abiotic environment acts as a habitat filter, permitting only those species with specific traits or ecological tolerances to co-occur (*1*, *2*), then the conservatism for hyphal length and root colonization in AMF could produce communities that consist only of species that are closely related to each other, or phylogenetically underdispersed (*2*, *8*–*10*). To determine whether this was the case, we sampled species richness in the old-field community from which the species pool of AMF was derived (*17*). We found that the community had species from all three AMF families, indicating that it was similar to our experimentally assembled species-rich communities (Fig. 2C). Thus, we conclude that the phylogenetically overdispersed experimental AMF communities we assembled were ecologically realistic.

Because of trait, and therefore niche, conservatism within the AMF (Fig. 2, A and B, and table S1) (*19*), our results suggest that the primary mechanism responsible for increased species richness in phylogenetically overdispersed communities is competitive exclusion preventing closely related and functionally similar species from co-occurring (*2*). Our results are therefore consistent with life-history and niche-based determinants of community assembly (*4*) as opposed to neutral models (*20*). Nevertheless, there is no clear consensus on the role of evolution in contemporary community assembly, in part because

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the degree of niche conservatism varies with the functional traits of interest (*21*) and the evolutionary and biogeographic history of a particular group (*3*). If niche conservatism is absent because natural selection favors ecological divergence among closely related taxa, the community and ecosystem consequences of phylogenetic relationships could be weak (*2*, *3*). However, if niche conservatism is widespread (*10*, *22*, *23*), the patterns we report here could occur in many communities, particularly in situations where species interact on fine spatial scales (*24*).

Our results have implications for understanding the mechanistic basis of the relationship between species richness and ecosystem functions such as productivity, nutrient cycling and resistance to disturbance (*25*, *26*). In particular, one mechanism that explains the positive relationship between species richness and ecosystem productivity is functional trait complementarity among co-occurring species (*27*). Although many experimental studies have observed support for this mechanism (*25*, *26*), these studies are open to the criticism that measured functional consequences are an artifact of experimental designs in which communities are randomly assembled and artificially maintained over time (*26*, *28*–*31*). For example, increased productivity at high species richness could be caused by the increased probability that species-rich communities will randomly contain an especially productive species (*28*, *29*). Moreover, the trait complementarity that maintains enhanced ecosystem function in an experimentally produced species-rich community may not be representative of that found in a natural community if these traits do not also promote stable coexistence among the same species (*31*). Thus, tests of the mechanisms that regulate the relationship between biodiversity and ecosystem function require that community assembly in experimental units reflect realistic, nonrandom ecological processes (*26*, *30*, *32*).

Our community assembly results indicate that such a test is possible in the AMF-plant system because two functional traits that contribute to enhanced plant productivity—protection from soil pathogens and increased plant uptake of nutrients (*33*, *34*)—appear to be conserved within an evolutionary lineage along with traits associated with spatial niche specialization. Our experiments (*17*) indicate that high root colonization by the Glomeraceae in comparison with other AMF families (Fig. 2A) is correlated with reduced root infection of *P. lanceolata* by two common soil pathogens (Fig. 3, A and B). In contrast, the high level of extraradical hyphal growth in the Gigasporaceae as compared with other AMF families (Fig. 2B) is correlated with enhanced P concentration in *P. lanceolata* shoots (Fig. 3C). If greater pathogen protection and enhanced P uptake are complementary, then plant productivity could be stimulated to a greater degree when both Glomeraceae and Gigasporaceae are in the same community. In contrast, the low root colonization (Fig. 2A) and short hyphal length (Fig. 2B) of the Acaulosporaceae may allow them to coexist with either the Glomeraceae or Gigasporaceae (Fig. 2C), but the low pathogen protection and low P-uptake capacity associated with these traits (Fig. 3, A to C) suggest that the Acaulosporaceae will not complement the function of the other two AMF families in an ecosystem.

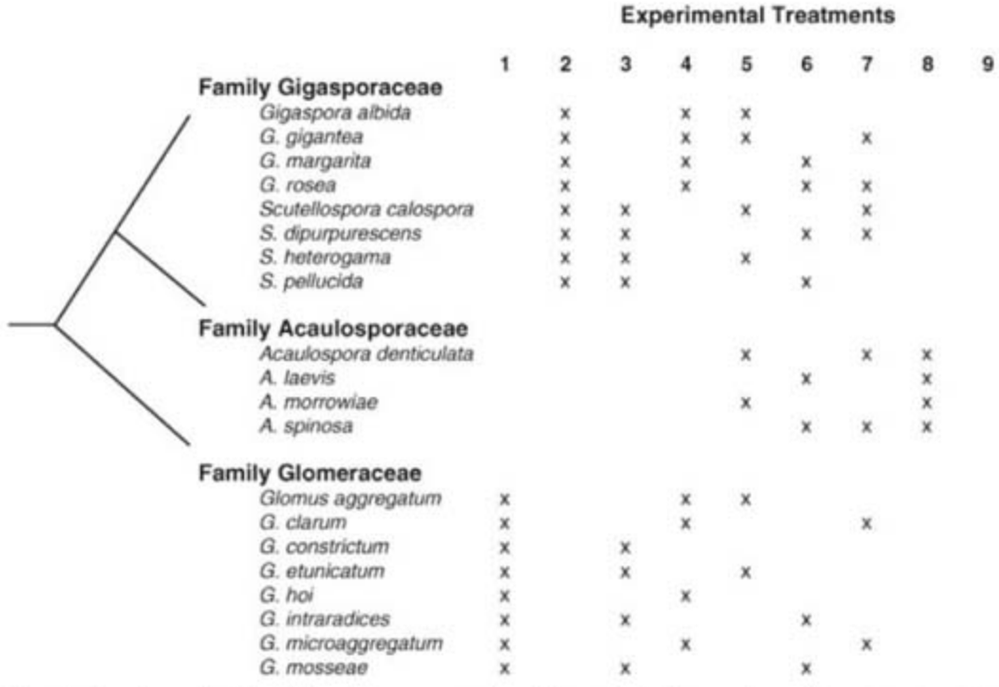

Fig. 1. Experimental design. Fungal taxa were assigned to each of the experimental treatments on the basis of their phylogenetically defined lineage. In treatments 1, 2, and 8, experimental units were constructed with species from one fungal family; treatments 3 and 4 with species from two fungal families; treatments 5 to 7 with species from three fungal families; and treatment 9 with no fungi.

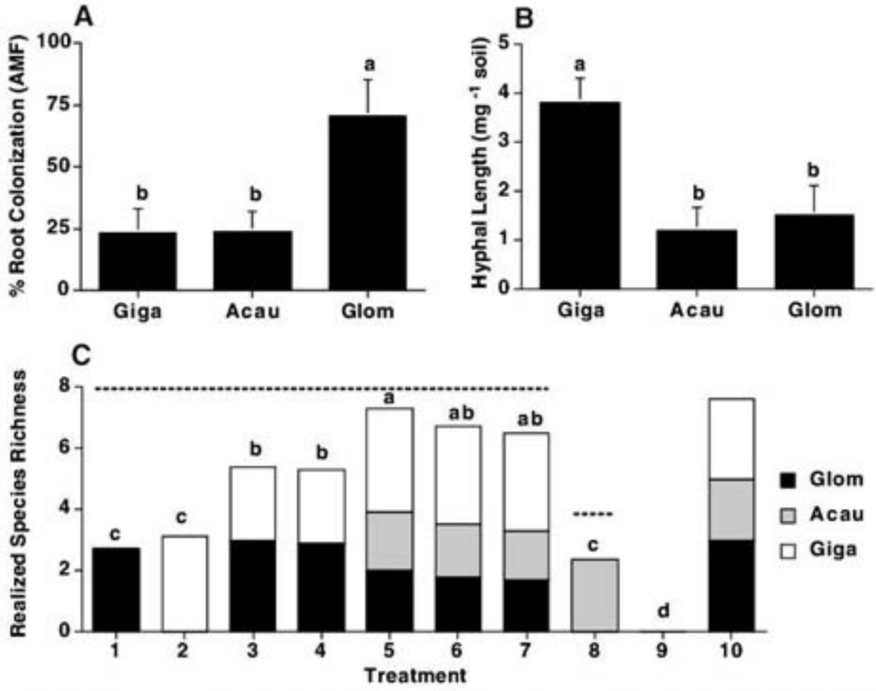

Fig. 2. Community assembly. The effect of different AMF species on (**A**) percent of root colonization by AMF (a measure of fungal growth inside the root) and (**B**) hyphal length (a measure of fungal growth outside the root). Each AMF species was grown in monoculture, but results are reported by fungal family (Giga, Gigasporaceae; Acau, Acaulosporaceae; Glom, Glomeraceae). Results by species are reported in table S1 (*17*). (**C**) The effect of phylogenetic dispersion of the fungal species pool on community assembly. Treatments are identified in Fig. 1 (number 10 represents the field study). The horizontal dashed lines represent the initial species richness. Different letters above each bar represent statistically significant differences ($P < 0.05$) after an analysis of variance (ANOVA) and a Tukey post hoc test.

We assessed one ecosystem consequence of variation in AMF species richness by measuring plant productivity (total biomass of individual *P. lanceolata*) in each community assembly treatment (Fig. 1) after 1 year of growth. *P. lanceolata* biomass was lowest in communities derived from phylogenetically underdispersed species pools. In fact, plant biomass did not differ significantly from that of nonmycorrhizal controls when grown with only a single AMF family (Fig. 3D). Plant biomass increased when the two putatively complementary AMF families (Glomeraceae and Gigasporaceae) were present in the community (Fig. 3D). In contrast, plant biomass was not stimulated by adding the third, putatively noncomplementary AMF family (Acaulosporaceae) to the experimental units. Plant biomass in communities derived from native soil was similar to that in the most productive experimentally assembled fungal communities (Fig. 3D). Therefore, our results also indicate that the effect of a natural field-derived AMF community on plant productivity was ecologically similar to that found in the overdispersed AMF treatment. The complementary effect of the different AMF families on ecosystem function was also supported by a strong positive relation between realized AMF species richness and plant productivity (Fig. 3E).

Most experimental tests of the effects of species richness on ecosystem functioning rely on randomly assembling communities and then maintaining the composition of that community over time (*26*, *30*, *31*). Because we allowed AMF communities to develop through a realistic ecological process based on niche conservatism, we could eliminate the role of artificially maintaining a high-diversity treatment (*31*) in a test for a positive relation between biodiversity and ecosystem functioning. Communities with high realized species richness only occurred when at least two lineages of AMF were present in the starting species pool (Fig. 2C). In addition, these communities contained the highest diversity of hyphal foraging capacity and pathogen protection, suggesting that enhanced plant productivity was caused by niche complementarity (*35*, *36*). Therefore, our explicit consideration of phylogenetic trait conservatism strengthens empirical support for the hypothesis that a positive relationship between diversity and ecosystem function is caused by increased functional trait richness (*25*–*27*, *30*, *31*, *35*).

Our results also suggest that phylogenetic relatedness can be a tool for predicting which species losses are most likely to negatively affect ecosystem functioning. For example, when species from multiple evolutionary lineages were replaced with an equal number of species from a single evolutionary lineage in experimental AMF communities, realized species richness and productivity declined. As a result, the functioning of this AMF/plant community is unlikely to be sensitive to species losses from within individual evolutionary lineages. However, the loss of an entire lineage could have strong negative ecological consequences. Our work therefore highlights the utility of information on phylogenetic relationships within communities to prioritize species conservation efforts aimed at maintaining important ecosystem functions and services (*37*).

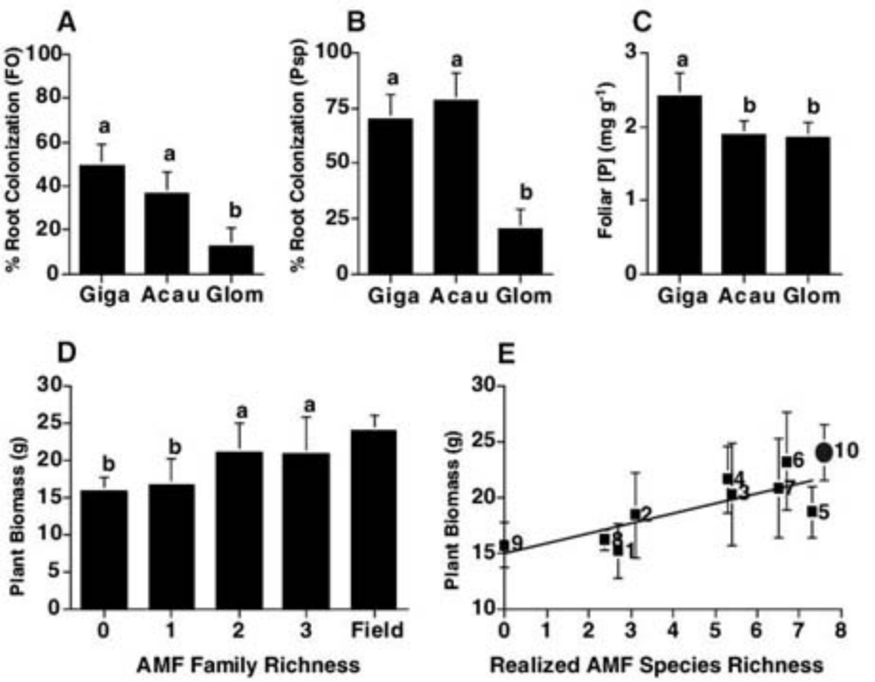

Fig. 3. Ecosystem functioning. The effect of different AMF species on (**A**) percent of root colonization by *Fusarium oxysporum* (FO) (root pathogen 1), (**B**) percent of root colonization by *Pythium* sp. (Psp) (root pathogen 2), and (**C**) foliar P concentration. Each AMF species was grown in monoculture, but results are reported by AMF family. Results by species are reported in table S1 (*17*). (**D**) The effect of phylogenetic dispersion (AMF family richness) and (**E**) realized AMF species richness on plant biomass ($y = 0.91x + 14.92$; $r^2 = 0.24$; $P = 0.001$). The numbers next to the boxes represent the experimental treatments in Fig. 1. The circle identified with number 10 represents the field study. Different letters above each bar in (A) to (D) represent statistically significant differences ($P < 0.05$) after an ANOVA and a Tukey post hoc test.

References and Notes

1. E. Weiher, P. A. Keddy, *Ecological Assembly Rules: Perspectives, Advances, Retreats* (Cambridge Univ. Press, Cambridge, 1999).
2. C. O. Webb, D. D. Ackerly, M. A. McPeek, M. J. Donoghue, *Annu. Rev. Ecol. Syst.* **33**, 475 (2002).
3. J. B. Losos *et al.*, *Nature* **424**, 542 (2003).
4. J. Silvertown, *Trends Ecol. Evol.* **19**, 605 (2004).
5. C. Darwin, *The Origin of Species by Means of Natural Selection* (Murray, London, 1859).
6. C. Elton, *J. Anim. Ecol.* **15**, 54 (1946).
7. R. MacArthur, R. Levins, *Am. Nat.* **101**, 377 (1967).
8. R. Tofts, J. A. Silvertown, *Proc. R. Soc. London Ser. B* **267**, 363 (2000).
9. C. O. Webb, *Am. Nat.* **156**, 145 (2000).
10. J. Cavender-Bares, D. D. Ackerly, D. Baum, F. A. Bazzaz, *Am. Nat.* **163**, 823 (2004).
11. E. Weiher, P. A. Keddy, *Oikos* **73**, 323 (1995).
12. M. R. Winston, *Am. Nat.* **145**, 527 (1995).
13. T. M. Anderson, M.-A. Lachance, W. T. Starmer, *Am. Nat.* **164**, 709 (2004).
14. J. Silvertown *et al.*, *Proc. R. Soc. London Ser. B* **273**, 39 (2006).
15. S. Y. Strauss, C. O. Webb, N. Salamin, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 5841 (2006).
16. C. O. Webb, J. B. Losos, A. A. Agrawal, *Ecology* **87**, S1 (2006).
17. Materials and methods are available as supporting materials on *Science* Online.
18. A. Schussler, D. Schwarzott, C. Walker, *Mycol. Res.* **105**, 1413 (2001).
19. M. M. Hart, R. J. Reader, *New Phytol.* **153**, 335 (2002).
20. S. P. Hubbell, *The Unified Neutral Theory of Biodiversity and Biogeography* (Princeton Univ. Press, Princeton, NJ, 2001).
21. D. D. Ackerly, *Am. Nat.* **163**, 654 (2004).
22. A. T. Peterson, J. Soberón, V. Sánchez-Cordero, *Science* **285**, 1265 (1999).
23. A. Prinzing, W. Durka, S. Klotz, R. Brandl, *Proc. R. Soc. London Ser. B* **268**, 2383 (2001).
24. N. G. Swenson, B. J. Enquist, J. Pither, J. Thompson, J. K. Zimmerman, *Ecology* **87**, 2418 (2006).
25. M. Loreau *et al.*, *Science* **294**, 804 (2001).
26. D. U. Hooper *et al.*, *Ecol. Monogr.* **75**, 3 (2005).
27. M. Loreau, A. Hector, *Nature* **412**, 72 (2001).
28. M. A. Huston, *Oecologia* **110**, 449 (1997).
29. D. A. Wardle, *Oikos* **87**, 403 (1999).
30. B. Schmid, J. Joshi, F. Schlapfer, in *The Functional Consequences of Biodiversity: Experimental Progress and Theoretical Extensions*, A. P. Kinzig, D. Tilman, S. Pacala, Eds. (Princeton Univ. Press, Princeton, NJ, 2002), vol. 33, pp. 120–150.
31. A. B. Pfisterer, J. Joshi, B. Schmid, M. Fischer, *Basic Appl. Ecol.* **5**, 5 (2004).
32. E. S. Zavaleta, K. B. Hulvey, *Science* **306**, 1175 (2004).
33. K. K. Newsham, A. H. Fitter, A. R. Watkinson, *Trends Ecol. Evol.* **10**, 407 (1995).
34. M. G. A. van der Heijden, T. R. Scheublin, *New Phytol.* **174**, 244 (2007).
35. M. G. A. van der Heijden *et al.*, *Nature* **396**, 69 (1998).
36. Y. Lekberg, R. T. Koide, J. R. Rohr, L. Aldrich-Wolfe, J. B. Morton, *J. Ecol.* **95**, 95 (2007).
37. F. Forest *et al.*, *Nature* **445**, 757 (2007).

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Materials and Methods
Table S1
References

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Nuclear Actin Regulates Dynamic Subcellular Localization and Activity of the SRF Cofactor MAL

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Actin, which is best known as a cytoskeletal component, also participates in the control of gene expression. We report a function of nuclear actin in the regulation of MAL, a coactivator of the transcription factor serum response factor (SRF). MAL, which binds monomeric actin, is cytoplasmic in many cells but accumulates in the nucleus upon serum-induced actin polymerization. MAL rapidly shuttles between cytoplasm and nucleus in unstimulated cells. Serum stimulation effectively blocks MAL nuclear export, which requires MAL-actin interaction. Nuclear MAL binds SRF target genes but remains inactive unless actin binding is disrupted. Fluorescence resonance energy transfer analysis demonstrates that the MAL-actin interaction responds to extracellular signals. Serum-induced signaling is thus communicated to nuclear actin to control a transcriptional regulator.

Small guanosine 5′-triphosphate (GTP)–binding proteins of the Rho family control the assembly of the actin cytoskeleton in response to extracellular signals. Activation of Rho leads to the accumulation of filamentous actin (F-actin) through both filament stabilization and de novo polymerization with concomitant depletion of cellular levels of monomeric actin (G-actin). In fibroblasts, Rho signaling regulates the subcellular localization and/or activity of MAL, a G-actin–binding SRF coactivator (*1*–*3*). Experiments with actin-binding drugs or actin overexpression have suggested that MAL activity responds to G-actin concentrations (*4*–*6*). Actin-binding drugs have distinct effects on MAL. Serum-induced nuclear accumulation of MAL and SRF activity is inhibited by latrunculin B (LatB), whereas drugs such as cytochalasin D (CD), swinholide A (SwA), and jasplakinolide induce MAL nuclear accumulation and SRF activation in the absence of signals (*1*, *4*). CD and SwA also disrupt MAL-actin interaction in immunoprecipitation and protein-affinity precipitation assays (*1*, *6*), but the role of actin binding in MAL regulation has remained unclear.

We first tested whether interaction with actin retains MAL in the cytoplasm or controls its continuous nucleocytoplasmic shuttling. In unstimulated cells, inactivation of the exportin Crm1 by its specific inhibitory drug leptomycin B (LMB) induced nuclear accumulation of MAL or MAL–green fluorescent protein (GFP), and this required the B2 region of MAL, a putative nuclear import signal (Fig. 1A and fig. S1). This shows that MAL continuously transits through the nucleus and allows investigation of the signaling requirements for its nuclear import in the absence of export. LMB-induced nuclear accumulation of MAL, but not control proteins, was inhibited by the G-actin–sequestering drug LatB and coexpression of C3 transferase, which irreversibly inactivates Rho, wild-type actin, and the nonpolymerizable actin $Arg^{62}\rightarrow Asp^{62}$ (R62D) mutant (*5*, *7*) (Fig. 1B and fig. S2). Thus, Rho and actin signaling control MAL nuclear import.

LMB-induced MAL-GFP nuclear accumulation was rapid, being effectively complete within 5 min (Fig. 1C and fig. S3) and indicating that basal MAL nuclear export rates must be very high to maintain its cytoplasmic localization (see below). Even the maximum rate of serum-induced MAL-GFP nuclear accumulation was less than this basal import rate, suggesting that increased nuclear import is not the major mechanism of MAL relocalization (Fig. 1C). CD and jasplakinolide, which activate SRF (*4*), induced MAL-GFP nuclear accumulation at a rate comparable to that of LMB (Fig. 1C and fig. S3).

To analyze export directly, we fused MAL to photoactivatable GFP (PAGFP) (*8*, *9*). Fluorescence was activated in the nucleus by focal-plane-restricted multiphoton excitation (*10*), and its subsequent decay measured (Fig. 2A and fig. S4). In resting cells, export of MAL-PAGFP was extremely rapid, with an apparent initial rate of 2.90% s^{-1} (probably an underestimate because the 10-s excitation period is comparable to the decay of nuclear fluorescence), and LMB-sensitive. Export was dramatically reduced after serum stimulation (0.48% s^{-1}) and almost completely inhibited by drugs that induce MAL nuclear accumulation and SRF activation (*1*, *4*), including CD, SwA, and jasplakinolide. MAL-GFP remained nuclear for several hours after serum stimulation; this reflects continued signaling, because MAL reaccumulated in the cytoplasm upon LatB treatment or after serum removal, with an initial rate comparable to that in serum-stimulated cells (Fig. 2B and fig. S5). Thus, nuclear export rather than import represents the major regulatory step in serum-induced nuclear accumulation of MAL.

We next studied the interaction between recombinant MAL and purified actin. Gel filtration resolved a complex with a relative molecular mass of 252,000 and an apparent stoichiometry of 1:3 (Fig. 2C and figs. S6 and S7). Complex formation was insensitive to LatB

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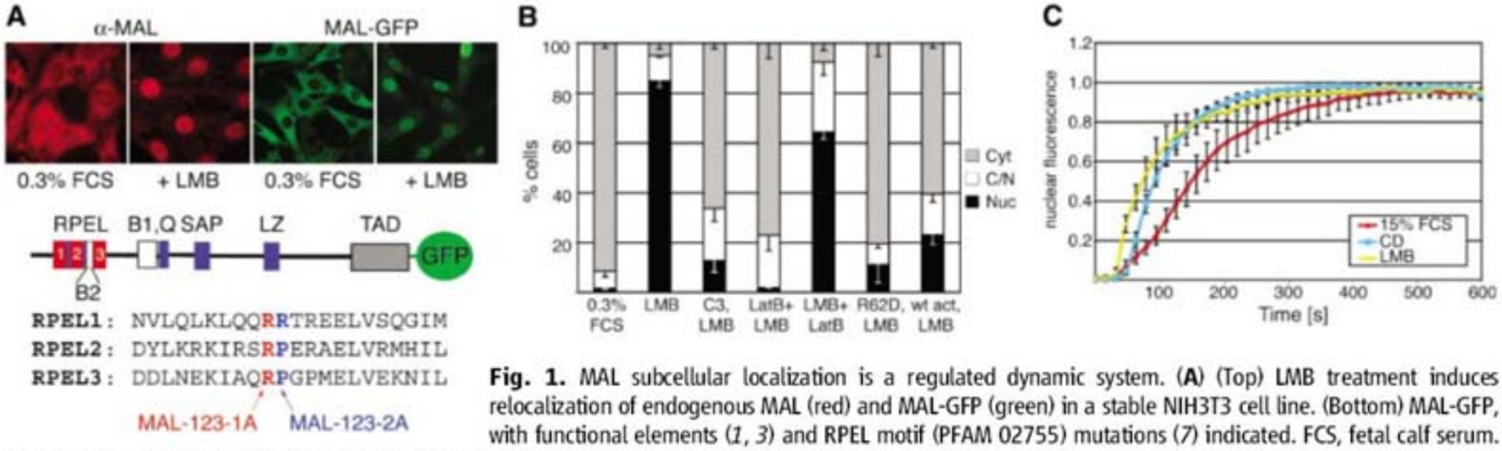


Fig. 1. MAL subcellular localization is a regulated dynamic system. (**A**) (Top) LMB treatment induces relocalization of endogenous MAL (red) and MAL-GFP (green) in a stable NIH3T3 cell line. (Bottom) MAL-GFP, with functional elements (*1*, *3*) and RPEL motif (PFAM 02755) mutations (*7*) indicated. FCS, fetal calf serum. (**B**) Rho-actin signaling is required for MAL nuclear import. Cyt, cytoplasmic; C/N, pancellular; Nuc, nuclear. (100 cells per point; $n = 3$ independent experiments; error bars indicate SEM). wt, wild type. (**C**) MAL rapidly accumulates in the nucleus (at least 12 cells per condition; error bars, SD).

but blocked by CD, SwA, or jasplakinolide. Substitution of the highly conserved positions 1 or 2 of each RPEL (*7*) motif with alanine (123-1A, 123-2A mutations, Fig. 1A) greatly reduced complex formation. Both gel filtration (Fig. 2C; see fig. S6 for further information) and a less-stringent glutathione *S*-transferase (GST)–MAL pulldown assay (Fig. 2D and fig. S7) indicated that MAL-123-2A exhibited somewhat greater residual affinity for actin than MAL-123-1A did. We used fluorescence loss in photobleaching (FLIP) (*8*) to compare the effect of RPEL mutations on MAL export with that of actin-binding drugs. MAL-123-1A and MAL-123-2A, which are nuclear in unstimulated cells, exhibited low export rates essentially identical to that of the wild-type protein in the presence of drugs that disrupt actin binding (Figs. 2E and 3B and fig. S8). Actin overexpression did not alter the subcellular localization of MAL-123-1A-GFP but slightly increased its export rate in the FLIP assay, which was prevented by CD (Fig. 2E and fig. S8). Actin overexpression redistributed MAL-123-2A to the cytoplasm, consistent with its greater residual affinity for actin, precluding analysis by FLIP (fig. S8). These data show that interaction with actin is required for Crm1-dependent MAL nuclear export.

Although it induced MAL nuclear accumulation, LMB treatment activated neither an SRF reporter nor transcription of the MAL-dependent SRF target genes *Vcl*, *Srf*, *Cyr61*, and *Acta2* in the absence of serum or CD stimulation (Fig. 3A and fig. S9), suggesting that disruption of actin-MAL interaction is required for nuclear MAL to activate SRF; the MAL-independent SRF target

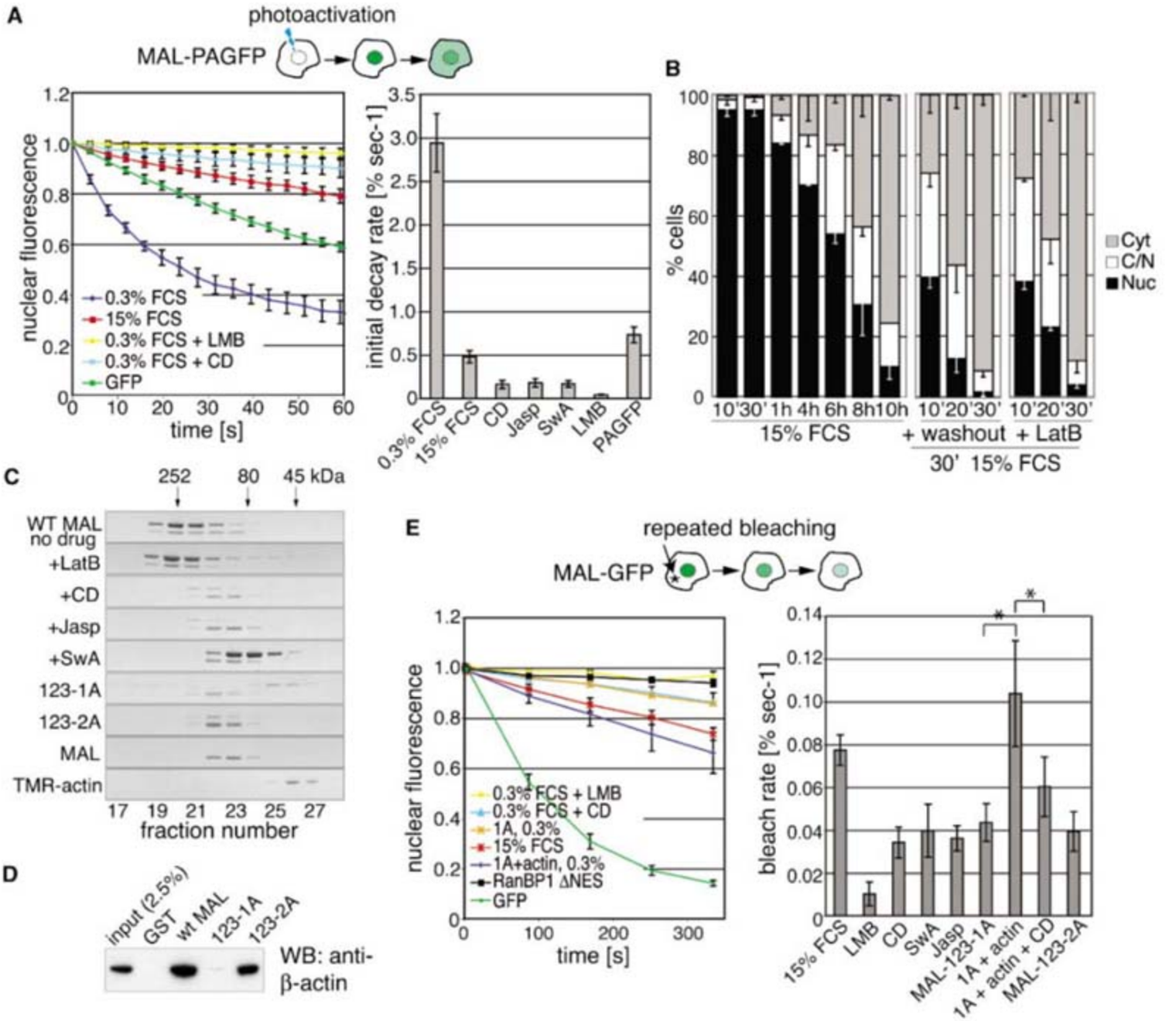


Fig. 2. Actin binding and nuclear export. (**A**) Serum stimulation decreases MAL nuclear export rate. Decay kinetics of nuclear fluorescence after MAL-PAGFP nuclear photoactivation (>10 cells per condition; error bars, SD). (**B**) Nuclear accumulation of MAL-GFP requires continuous signaling. MAL-GFP localization after serum stimulation with or without additional serum washout and LatB treatment. h, hours. $n = 3$; error bars, SEM. (**C**) Sensitivity of a stable MAL-actin complex to actin-binding drugs and RPEL mutations. RPEL domain was bound to G-actin, and apparent molecular masses analyzed by gel filtration. Note that SwA dimerizes actin. Further details are in figs. S6 and S7. (**D**) GST affinity precipitation analysis of MAL-actin interaction. WB, Western blot. GST baits are shown in fig. S7. (**E**) Nuclear export requires interaction with actin. Nuclear export rates of wild-type or mutant MAL-GFP proteins measured by FLIP assay, quantified as in (A) (Student's t test, $^*P < 0.05$). Error bars, SEM.

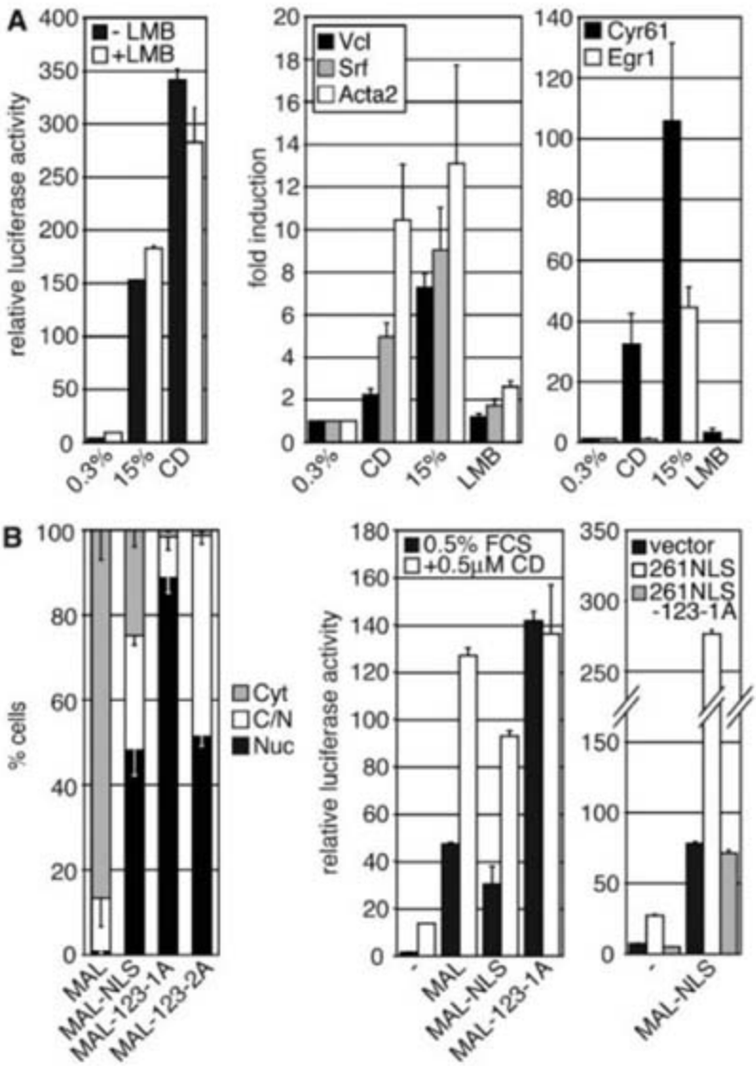
gene Egr1 was unaffected. Consistent with this, CD potentiated activation of an SRF reporter by overexpression of MAL-NLS, which contains a heterologous nuclear import signal and is substantially nuclear-localized, but not activation by the constitutively nuclear MAL-123-1A mutant, which cannot bind actin (Fig. 3B and figs. S8 and S10). MAL-NLS activity was also potentiated by nuclear co-expression of the wild-type MAL RPEL domain (MAL2-261-NLS) but not by its 123-1A derivative, suggesting that MAL-NLS is repressed by actin (Fig. 3B and fig. S10). Consistent with these data, we previously found that NLS-actin expression relocalizes MAL to the nucleus but represses SRF activity (*1*, *5*).

Together these results show that actin, or an actin-dependent cofactor, can repress MAL activity in the nucleus. This appears to occur at the level of gene activation rather than at DNA binding, because in chromatin immunoprecipitation experiments LMB treatment induced a substantial specific increase of MAL recruitment to its target genes *Vcl*, *Cyr61*, and *Srf*, comparable to that induced by CD or serum (Fig. 3C and fig. S11). Nuclear actin might recruit repressors to actin-MAL-SRF complexes or prevent recruitment of transcriptional co-activators. Previous studies have implicated actin in transcriptional control through regulation of RNA polymerases and chromatin-modification and -remodelling complexes (*11*, *12*).

To gain direct insight into actin-MAL interactions in cells, we exploited fluorescence resonance energy transfer (FRET), detected by fluorescence lifetime imaging (*13*). MAL-GFP was used as donor, and Cy3-labeled anti-myc, recognizing co-expressed Myc-actin, as acceptor. Under the assay conditions, the SRF reporter gene remained regulated (fig. S12). In unstimulated cells, FRET was readily detectable between MAL and actin, indicating that they physically interact (Fig. 4A and fig. S13). Treatment with CD reduced this interaction to background level, whereas LatB treatment increased it, consistent with biochemical and functional data (*1*, *6*). In contrast, no FRET was detected between MAL-123-1A and actin. Serum stimulation transiently reduced but did not abolish FRET between wild-type MAL and actin, which returned to its prestimulation level by 30 min (Fig. 4B). Similar results were observed with a MAL-GFP derivative lacking the B2 region, which remained cytoplasmic (Fig. 4B and fig. S1). In contrast, although LMB treatment induced MAL nuclear accumulation in unstimulated cells, it did not affect actin-MAL FRET, which could nevertheless be reduced to background level by CD treatment (Fig. 4A and fig. S12). In LMB-pretreated cells, serum stimulation also transiently reduced but did not abolish nuclear actin-MAL FRET, although recovery was slower than in untreated cells (Fig. 4B; see below). Thus, even when MAL is artificially confined to the nucleus, actin-MAL interaction can respond to serum-induced signals.

In serum-stimulated cells, the reduced but significant actin-MAL FRET, detectable when MAL is entirely nuclear, must reflect generation of a subpopulation of actin-free MAL or reduced actin-binding stoichiometry. It is likely that this reduced interaction at least initially reflects a rapid drop in the availability of G-actin, because total cellular deoxyribonuclease I (DNaseI)–stainable actin exhibited a similarly rapid decrease, followed by a slower recovery to prestimulation level (Fig. 4C). Actin thus interacts with MAL in both nucleus and cytoplasm, and serum-induced signals and actin-binding drugs change this interaction in a way consistent with the functional data. Together, these data provide direct support for our proposal that MAL activation reflects reduced MAL-actin interaction arising from depletion of the cellular G-actin pool (*1*).

We have demonstrated that actin regulates MAL activity at three levels: nuclear import,



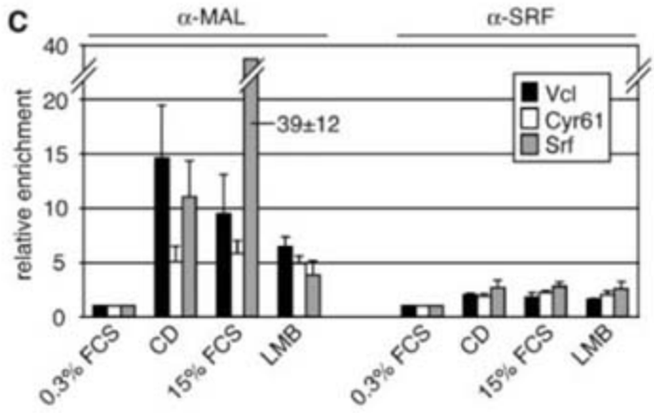


Fig. 3. Nuclear actin represses MAL activity. (**A**) LMB-induced MAL nuclear accumulation does not activate transcription of the SRF reporter (left) or endogenous SRF gene targets (right), measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR). 0.3%, serum-starved cells; 15%, serum-starved cells. $n = 3$; error bars, SEM. (**B**) Disruption of actin binding allows SRF activation by nuclear MAL derivatives. (Left) Localization of MAL derivatives in serum-starved cells. (Right) Activation of MAL-NLS by CD or overexpressed nuclear RPEL domain (261NLS) after 2-day starvation. (**C**) MAL nuclear accumulation allows association with target genes, measured by qPCR analysis of chromatin immunoprecipitates. $n = 3$; error bars, SEM.

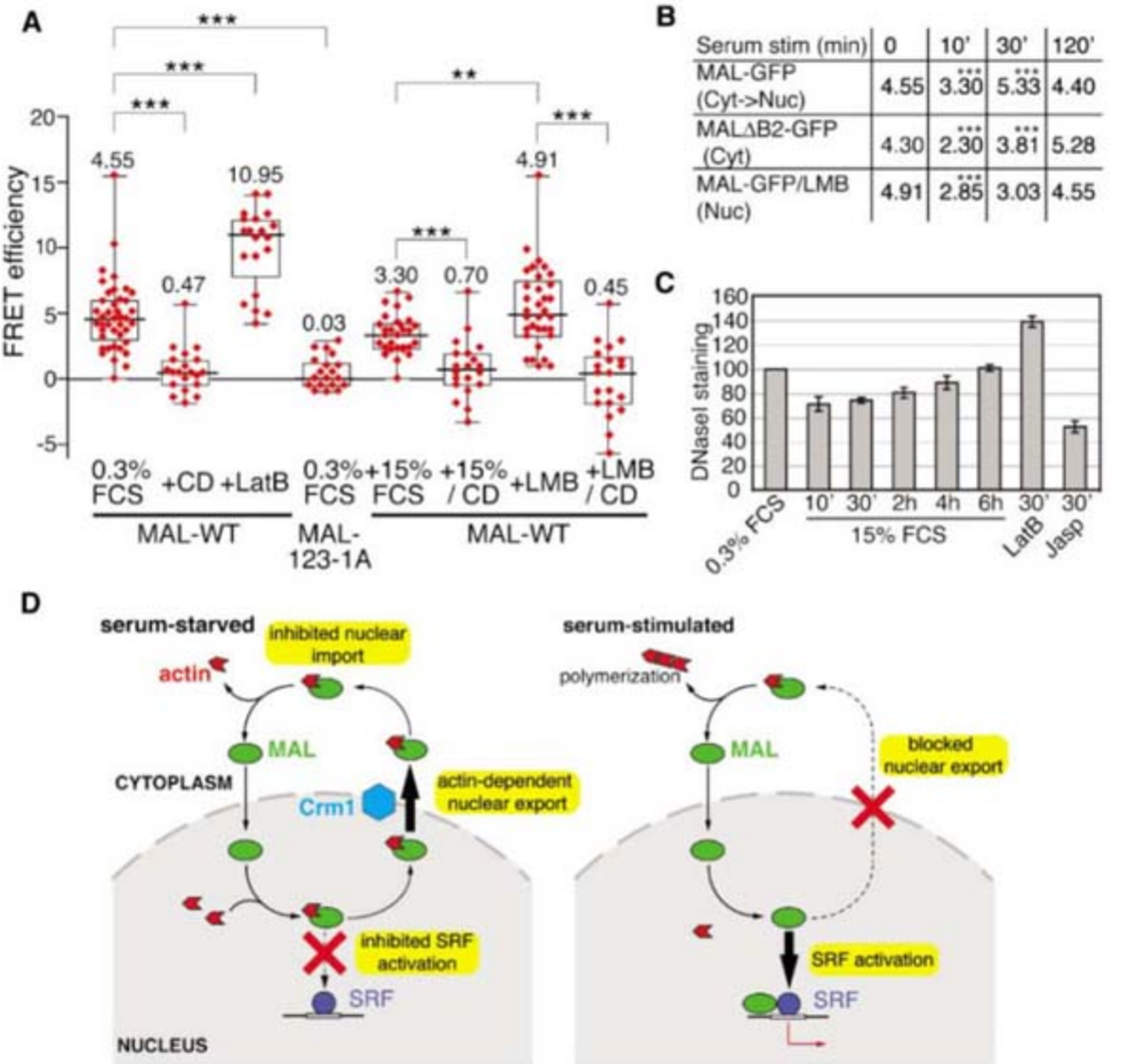


Fig. 4. MAL interacts with actin in both cytoplasm and nucleus. (**A**) Detection of MAL-actin interaction in vivo by FRET and FLIM. FRET efficiencies shown as box-and-whisker plots with median (>20 cells per condition; Mann-Whitney test, ***$P < 0.0005$; **$P < 0.005$). (**B**) Serum stimulation transiently decreases median FRET efficiency (***$P < 0.0005$ relative to previous point). (**C**) Serum stimulation transiently reduces DNaseI-stainable G-actin ($n = 3$; error bars, SD). (**D**) Multiple roles for actin in MAL regulation. In unstimulated cells, high export rates ensure MAL is mainly cytoplasmic, whereas nuclear actin prevents SRF activation. Upon stimulation, decreased export induces nuclear MAL accumulation, and diminished interaction with actin allows SRF activation. Proteins are shown as monomers for simplicity.

nuclear export, and activation of target gene transcription (Fig. 4D). Nuclear actin plays an especially important role in regulation of MAL localization and activity. MAL shuttles continuously between cytoplasm and nucleus, even in unstimulated cells. Serum-induced signals primarily control MAL subcellular localization by reducing the rate of its nuclear export, which requires actin binding and Crm1. However, our preliminary data indicate that recombinant RPEL domain–Crm1 interaction in vitro does not require actin. Perhaps actin instead facilitates interaction of Crm1-MAL complexes with nucleoporins (*14*). Defective nuclear export might explain the ability of some actin mutants to induce MAL nuclear accumulation even though they bind MAL effectively (*6*). Actin overexpression can also inhibit MAL nuclear import, perhaps by masking the B2 nuclear import signal, although this does not appear to play a major point of regulation in the cells studied here. It remains to be seen whether actin enters or exits the nucleus while bound to MAL.

Our data suggest that there must be communication between the cytoplasmic and nuclear actin pools. Other nuclear functions of actin may therefore be sensitive to extracellular signals. Actin-profilin complexes are subject to apparently constitutive export to the cytoplasm via interaction with exportin 6 (*15*), but the mechanisms of actin nuclear import remain obscure, and MAL itself may contribute. It will be interesting to assess how the dynamics of cytoplasmic-nuclear shuttling of actin impinge on MAL subcellular localization and activity in different cell types.

References and Notes

1. F. Miralles, G. Posern, A.-I. Zaromytidou, R. Treisman, *Cell* **113**, 329 (2003).
2. B. Cen *et al.*, *Mol. Cell. Biol.* **23**, 6597 (2003).
3. G. C. Pipes, E. E. Creemers, E. N. Olson, *Genes Dev.* **20**, 1545 (2006).
4. A. Sotiropoulos, D. Gineitis, J. Copeland, R. Treisman, *Cell* **98**, 159 (1999).
5. G. Posern, A. Sotiropoulos, R. Treisman, *Mol. Biol. Cell* **13**, 4167 (2002).
6. G. Posern, F. Miralles, S. Guettler, R. Treisman, *EMBO J.* **23**, 3973 (2004).
7. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr.
8. J. Lippincott-Schwartz, G. H. Patterson, *Science* **300**, 87 (2003).
9. G. H. Patterson, J. Lippincott-Schwartz, *Science* **297**, 1873 (2002).
10. B. Schmierer, C. S. Hill, *Mol. Cell. Biol.* **25**, 9845 (2005).
11. F. Miralles, N. Visa, *Curr. Opin. Cell Biol.* **18**, 261 (2006).
12. T. Pederson, U. Aebi, *Mol. Biol. Cell* **16**, 5055 (2005).
13. P. Wu, L. Brand, *Anal. Biochem.* **218**, 1 (1994).
14. D. Engelsma, R. Bernad, J. Calafat, M. Fornerod, *EMBO J.* **23**, 3643 (2004).
15. T. Stuven, E. Hartmann, D. Gorlich, *EMBO J.* **22**, 5928 (2003).

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Quantitative Morphological Signatures Define Local Signaling Networks Regulating Cell Morphology

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Although classical genetic and biochemical approaches have identified hundreds of proteins that function in the dynamic remodeling of cell shape in response to upstream signals, there is currently little systems-level understanding of the organization and composition of signaling networks that regulate cell morphology. We have developed quantitative morphological profiling methods to systematically investigate the role of individual genes in the regulation of cell morphology in a fast, robust, and cost-efficient manner. We analyzed a compendium of quantitative morphological signatures and described the existence of local signaling networks that act to regulate cell protrusion, adhesion, and tension.

Morphogenesis commonly relies on the spatial and temporal regulation of distinct groups of genes acting in local signaling networks. The morphology of a single cell also results from the spatiotemporally regulated activity of signaling proteins. For example, Rac-type guanosine triphosphatases (GTPases) promote the formation of protrusive lamellipodia at the leading edge of motile cells, whereas Rho-type GTPases promote cortical tension and cell retraction at the rear of the cell through the activation of the actomyosin machinery (*1*). Both protrusive activity and cell body retraction are tightly coupled to the assembly and disassembly of adhesive structures through Rho signaling (*2*). Many signaling proteins must act both upstream and downstream of specific Rho GTPases in spatially distinct subcellular local networks to translate extracellular signals to changes in GTPase activation and ultimately in cellular morphology. However, the components of these networks and the precise role they play in regulating cell shape remain largely unclear.

We performed a genetic screen of 249 gene-overexpression or double-stranded RNA (dsRNA) treatment conditions (TCs) using the *Drosophila* BG-2 cell line to determine the roles of genes acting in local networks to control distinct aspects of cell morphology. BG-2 cells are highly motile and exhibit many of the traits observed in mammalian fibroblasts and epithelial cells, including the formation of integrin-based adhesions, polarized lamellipodia, and coordinated retraction of the cell body (*3*, *4*); but unlike many mammalian cell types, the growth of BG-2 cells is not inhibited by contact with other cells. To analyze the morphologies of cells in each TC, we used protocol and image-processing techniques designed to detect clear and complete boundaries of individual cells and to quantitatively analyze the shapes of these boundaries along with the intensities and textures of their interiors. First, we stochastically labeled samples with green fluorescent protein (GFP) [supporting online material (SOM)] to enable individual cells to stand out in the crowded and overgrown samples, acquired images of these cells using conventional fluorescence microscopy, and used software to identify the boundaries of individual cells (Fig. 1A and SOM). Although these techniques make lower numbers of cells per sample available to subsequent analysis as compared to other published methods (*5*, *6*), we preferred them because they yielded detailed, high-quality empirical boundaries instead of algorithmically determined approximations. For each individual cell, we computed 145 different quantitative features that reflected basic aspects of cell geometry, detailed aspects of cellular protrusions, or the distribution of GFP intensity within the cellular boundaries (Fig. 1B and SOM). Altogether we analyzed 12,601 individual cells from our 249 TCs.

To transform our 145 features into biologically meaningful morphological indicators, we trained a set of neural networks (NNs) to use informative subsets of the features to discriminate cells from particular reference TCs from sets of other reference TCs (Fig. 1D and SOM). We targeted seven TCs for NN training because they produced phenotypes that were qualitatively distinctive and discernable from control cells (SOM). For example, overexpression of an N-terminally truncated form of the Rho guanine nucleotide exchange factor (RhoGEF) SIF (ΔN-SIF), the *Drosophila* ortholog of mammalian Tiam-1, stimulates extensive lamellipodia formation, cell spreading, and a general loss of tension, demonstrated by the flat and thin appearance of the ΔN-SIF cells (Fig. 2) (*7*). After training a ΔN-SIF NN to distinguish ΔN-SIF cells from the cells of our six other target TCs, we applied this NN to all 12,601 cells in our data set to score each of them for this distinctive morphology (Fig. 1D). In addition to ΔN-SIF, we trained NNs for TCs treated with overexpression constructs for RacV12, RacF28L, RhoV14, RhoF30L, CG3799 full-length, and ΔN-RhoGEF3. The seven TCs selected for NN training included several for which the underlying mechanisms responsible for their phenotypes are not understood.

Finally, for each NN and TC, we calculated a NN *Z* score (NNZ), which is the variance-adjusted difference between the mean NN score of all cells in the TC and the mean NN score of all cells in our data set. Each NNZ is thus an index of the morphology of an entire TC (SOM) (Fig. 1E). For example, for cells in which CG10188 (a RhoGEF) was targeted by dsRNA, the NNZ for the ΔN-SIF NN was 0.76, which indicates that CG10188 dsRNA induces a morphology that is slightly more "ΔN-SIF–like" than an equal number of randomly chosen cells (which would have a NNZ of 0). In contrast, the NNZ for ΔN-SIF cells using the ΔN-SIF classifier is 26.77. Together, the seven NNZs computed for each TC constituted a quantitative morphological signature (QMS) of the TC (Fig. 1E). A QMS is thus a high-order representation of the morphology of cells in a TC as a vector of seven specific quantitative similarities and dissimilarities with seven panels of reference cells with distinctive phenotypes.

Two-dimensional hierarchical clustering (SOM) of 249 QMSs revealed that TCs, and in particular RNA interference (RNAi) against individual genes, fell into several distinct clusters. QMSs with similar qualitative phenotypes clustered tightly together (Fig. 2). We define "phenoclusters" as genes grouped at the highest node in the clustering for which the cluster distance metric (an average of uncentered Pearson correlation coefficients) was greater than 0.80 and term this a "cluster distance cutoff" (CDC) (Fig. 2) (*8*). A value of 0.80 was chosen as the CDC because smaller cutoffs resulted in groupings of visually diverse morphologies, whereas higher thresholds resulted in the segregation of visually similar morphologies into distinct clusters.

A large phenocluster was composed of TCs that clustered because their QMSs have high ΔN-RhoGEF3 NNZs (cluster 6). All cells in this cluster were extremely round and had very few or no protrusions of any type (Fig. 2 and fig. S18). QMSs for *p190RhoGAP*, *SCAR*, *slingshot*, *armadillo*, *ankyrin*, *Sop2*, and *RhoGEF3* RNAi were clustered together. Moreover, we observed an enrichment in this cluster for RNAi against genes involved in Rap signaling (three of six genes in the data set). The finding that depletion of either SCAR, the cofilin phosphatase Slingshot, or Sop2 resulted in defects in protrusion is consistent with the known function of these three proteins (*4*, *9*–*14*). Gef26 and its downstream target Rap1 function in the formation of adherence junctions in *Drosophila* (*15*–*17*) and have recently been observed to be required for cell spreading and migration of *Drosophila* macrophages (*18*). In mammalian systems, p190RhoGAP acts downstream of integrins and adhesions to promote cell

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spreading (*19*). Thus, our method successfully identified two distinct but coupled signaling pathways that regulate the formation of protrusions. We anticipate that *Drosophila* RhoGEF3 plays a critical role in the regulation of adhesion, because overexpression of a N-terminally deleted form or inhibition by dsRNA have similar QMSs. ΔN-RhoGEF3 is not likely to be constitutively activated (*20*) but may promote cell rounding by acting in a dominant-negative manner toward endogenous RhoGEF3 signaling.

A second phenocluster contained a group of TCs that co-clustered with RhoF30L and had high RhoF30L NNZs (cluster 8). These cells differed qualitatively from cells in the ΔN-RhoGEF3 phenocluster by virtue of the fact that cells in this cluster did have some visible, but poorly formed, lamellipodial protrusions (Fig. 2 and fig. S18). dsRNAs in this cluster may target genes that specifically promote the formation of lamellipodia, and RhoF30L (an activated form of Rho that cycles between GTP- and GDP-bound states) may inhibit this process. In support of this notion, dsRNAs targeting *twinstar*, *capt*, and *ARC-p20* were part of this cluster, representing an enrichment in genes that have been identified in previous screens for genes required for lamellipodia organization (three of seven genes) (*10*). Given the fact that lamellipodia formation occurs after the formation of adhesion, protrusion, and actin-filament nucleation, this suggests that phenotypic profiling can not only simultaneously monitor the activity of coupled signaling pathways (within phenoclusters) but can also monitor the temporal hierarchical relationships that exist among local signaling networks.

The largest phenocluster was a group of TCs that shared high ΔN-SIF, RacV12, and RacF28L NNZs (cluster 18) and that corresponded to large flat cells, typically with extensive lamellipodia (Fig. 2 and fig. S18). This phenotype is consistent with repeated observations of cells overexpressing activated Rac mutant proteins or activated RacGEFs such as SIF or Tiam-1 (*7*, *21*). QMSs for *cenG1A*, *cenB1A*, *CG16728*, and *CG13692* RNAi were members of this phenocluster, representing an enrichment in ArfGAPs (four of six genes). Mammalian ArfGAPs such as GIT1 and

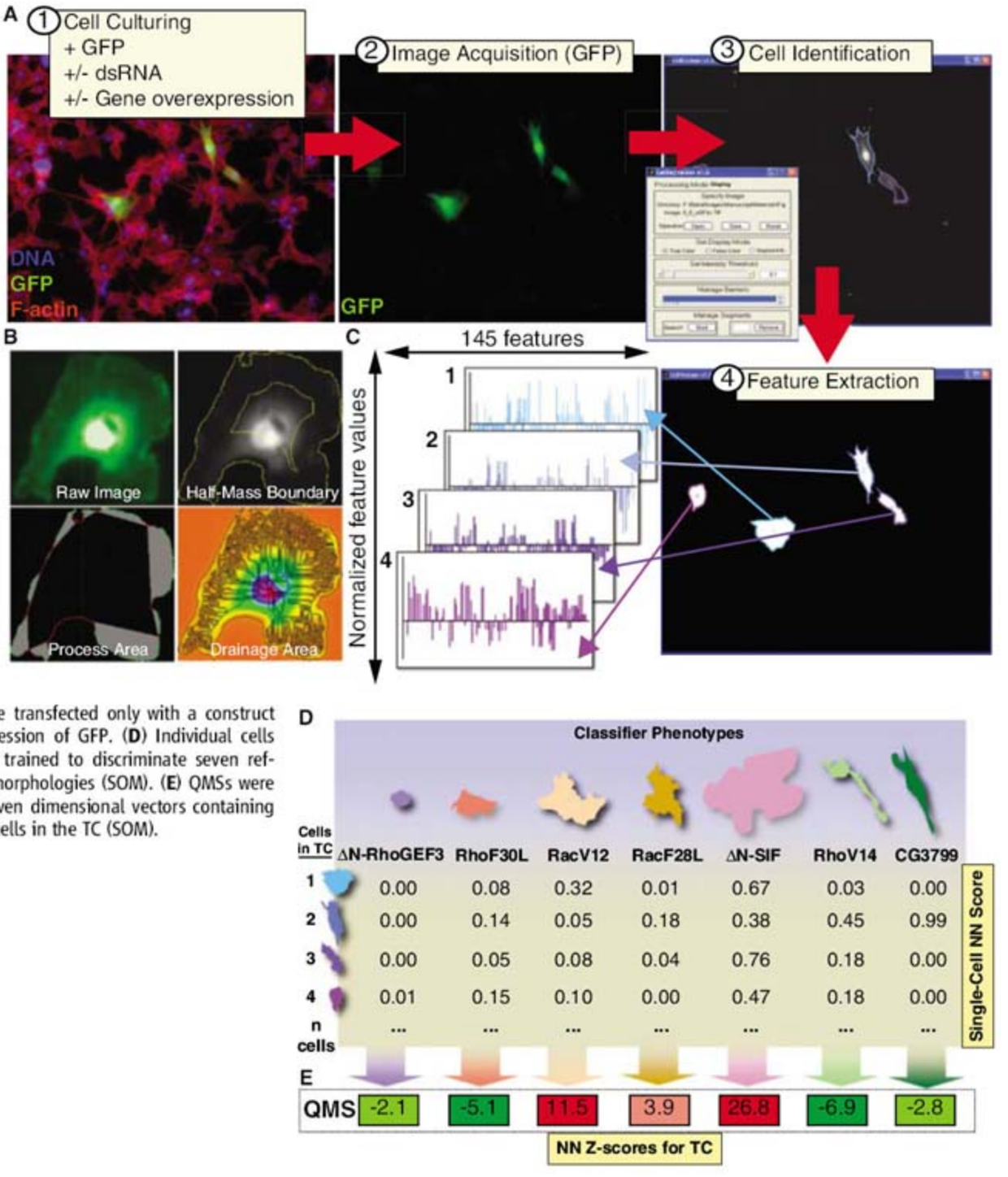

Fig. 1. Phenotypic profiling workflow. (**A**) Cultured *Drosophila* BG-2 cells were transfected with plasmids encoding GFP and either cotransfected with plasmids encoding red fluorescent protein–tagged proteins or incubated in the presence of dsRNA for 4 days. Images of GFP-labeled cells were acquired by standard fluorescence microscopy, and individual cell images with clear and complete boundaries were selected with custom-developed software (SOM). (**B**) Graphical representations of some of the features computed from each individual cell image (SOM). (**C**) 145 different features relevant to cell morphology and GFP signal intensity were derived from individual cells and expressed as *Z* scores relative to their values over a subset of GFP control cells which were transfected only with a construct coding for constitutive expression of GFP. (**D**) Individual cells were then scored with NNs trained to discriminate seven reference TCs with distinctive morphologies (SOM). (**E**) QMSs were computed for each TC as seven dimensional vectors containing the NNZs of the individual cells in the TC (SOM).

GIT2 promote the disassembly of integrin-based focal adhesions by binding the tyrosine-phosphorylated form of Paxillin, which in turn results in ArfGTPase activation and a concomitant down-regulation of Rac activity and adhesion turnover (*22*–*24*). In addition to ArfGAPs, this phenocluster also contained QMSs for dsRNAs targeting *paxillin* (fig. S18) and α-actinin, and was defined by high levels of Rac activity (Fig. 2). Based on this and sequence analysis, we suggest that *CG16728* is the *Drosophila* ortholog of mammalian GIT ArfGAPs. Proteomic analysis has recently revealed that GIT1 is part of a supramolecular complex that, in addition to Paxillin, includes β_2 centaurin and gelsolin and directly binds moesin (*25*). *Drosophila cenB1A* (the *Drosophila* ortholog of β_2 *centaurin*), *Gelsolin*, and *Moesin* dsRNA were also members of same phenocluster as *CG16728* and *paxillin*, further demonstrating that our methodology is capable of identifying both functionally and physically coupled signaling components. Taken together, this suggests that the large, flat, and spread morphology of cells in this phenocluster was partially due to inhibiting the disassembly of adhesion and that QMSs can be used to functionally annotate genes.

We have demonstrated that quantitative morphological profiling of single cells combined with RNAi-based genetic screening technology results in the identification of local signaling networks with spatially, temporally, and functionally defined characteristics that act in a hierarchical manner to regulate cell shape and migration. These methods can be used not only in the context of genetic screens but also in large-scale screens of small-molecule libraries or screens involving the overexpression of cDNAs. Because our approach is a fast and cost-effective way to query the activity of multiple signaling proteins and pathways, quantitative morphological profiling may also be useful as a diagnostic tool in the analysis of clinical samples. Furthermore, akin to gene-expression data, we can now use morphological phenotypic data for computa-

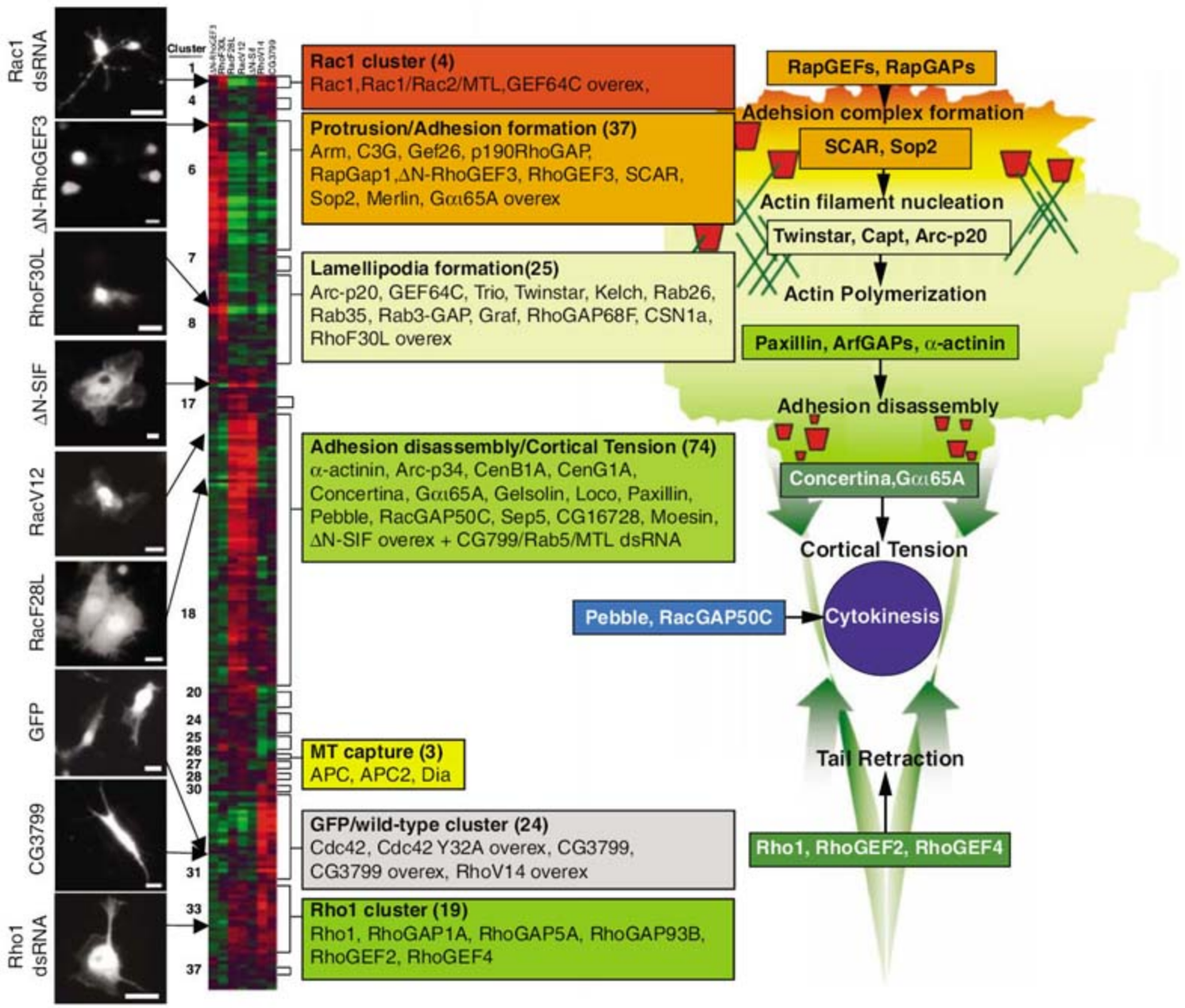

Fig. 2. Identification of local networks that regulate distinct aspects of morphology. Hierarchical clustering of the genes in the data set (the *y* axis) by how cells scored on the ΔN-SIF, ΔN-RhoGEF3, CG3799, RacF28L, RacV12, RhoF30L, and RhoV14 NNs (the *x* axis) is shown. We define phenoclusters of genes as clusters with a CDC >0.80, which results in 41 total clusters comprising 17 multigene clusters and 24 singletons. All multigene clusters are identified in brackets on the right-hand side of the clustergram. For some clusters, we describe prominent TCs, and the number of TCs within these clusters is indicated in parentheses. Examples of individual cells and their positions in the clustergram are shown on the left-hand side of the clustergram. Based on their gene membership, a number of clusters were determined to have specialized roles in cell morphology. A complete listing of clusters is provided in table S8. Scale bars, 10 μm.

tional approaches that aim to model the dynamic nature of signaling networks, while the RNAi component pushes us closer to causal mechanistic linkages.

References and Notes

1. A. Hall, *Biochem. Soc. Trans.* **33**, 891 (2005).
2. K. A. DeMali, K. Wennerberg, K. Burridge, *Curr. Opin. Cell Biol.* **15**, 572 (2003).
3. Y. Takagi, K. Ui-Tei, T. Miyake, S. Hirohashi, *Neurosci. Lett.* **244**, 149 (1998).
4. A. Biyasheva, T. Svitkina, P. Kunda, B. Baum, G. Borisy, *J. Cell Sci.* **117**, 837 (2004).
5. Z. E. Perlman *et al.*, *Science* **306**, 1194 (2004).
6. A. E. Carpenter *et al.*, *Genome Biol.* **7**, R100 (2006).
7. M. Sone *et al.*, *Science* **275**, 543 (1997).
8. K. C. Gunsalus *et al.*, *Nature* **436**, 861 (2005).
9. J. A. Zallen *et al.*, *J. Cell Biol.* **156**, 689 (2002).
10. S. L. Rogers, U. Wiedemann, N. Stuurman, R. D. Vale, *J. Cell Biol.* **162**, 1079 (2003).
11. P. Kunda, G. Craig, V. Dominguez, B. Baum, *Curr. Biol.* **13**, 1867 (2003).
12. N. Zebda *et al.*, *J. Cell Biol.* **151**, 1119 (2000).
13. M. Nishita *et al.*, *J. Biol. Chem.* **279**, 7193 (2004).
14. M. D. Welch, A. H. DePace, S. Verma, A. Iwamatsu, T. J. Mitchison, *J. Cell Biol.* **138**, 375 (1997).
15. A. L. Knox, N. H. Brown, *Science* **295**, 1285 (2002).
16. H. Wang *et al.*, *Dev. Cell* **10**, 117 (2006).
17. S. R. Singh *et al.*, *Dev. Growth Differ.* **48**, 169 (2006).
18. S. Huelsmann, C. Hepper, D. Marchese, C. Knoll, R. Reuter, *Development* **133**, 2915 (2006).
19. W. T. Arthur, K. Burridge, *Mol. Biol. Cell* **12**, 2711 (2001).
20. K. Murayama *et al.*, *J. Biol. Chem.* (26 December 2006).
21. E. E. Sander, J. P. ten Klooster, S. van Delft, R. A. van der Kammen, J. G. Collard, *J. Cell Biol.* **147**, 1009 (1999).
22. D. J. Webb *et al.*, *Nat. Cell Biol.* **6**, 154 (2004).
23. M. C. Brown, L. A. Cary, J. S. Jamieson, J. A. Cooper, C. E. Turner, *Mol. Biol. Cell* **16**, 4316 (2005).
24. N. Nishiya, W. B. Kiosses, J. Han, M. H. Ginsberg, *Nat. Cell Biol.* **7**, 343 (2005).
25. M. W. Mayhew *et al.*, *J. Proteome Res.* **5**, 2417 (2006).

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Supporting Online Material

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Restriction of an Extinct Retrovirus by the Human TRIM5α Antiviral Protein

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Primate genomes contain a large number of endogenous retroviruses and encode evolutionarily dynamic proteins that provide intrinsic immunity to retroviral infections. We report here the resurrection of the core protein of a 4-million-year-old endogenous virus from the chimpanzee genome and show that the human variant of the intrinsic immune protein TRIM5α can actively prevent infection by this virus. However, we suggest that selective changes that have occurred in the human lineage during the acquisition of resistance to this virus, and perhaps similar viruses, may have left our species more susceptible to infection by human immunodeficiency virus type 1 (HIV-1).

A large portion of primate genomes is composed of endogenous retroviruses that can be thought of as an archaeological record of past infections. Both chimpanzee and gorilla genomes harbor more than 100 copies of *Pan troglodytes* endogenous retrovirus (PtERV1), whereas it is absent from the human genome (*1*). Comparison of individual PtERV1 proviruses in gorilla and chimpanzee genomes suggest that this virus was active 3 to 4 million years ago, after the separation of chimpanzee and human lineages (*1*). This raises an evolutionary conundrum as to why sister species, but not humans, acquired germline copies of this retrovirus even though all three species cohabited when PtERV1 was an active exogenous virus (*1*). One mechanism of active restriction from retroviral infections is conferred by the TRIM5α protein, which binds directly to the incoming retroviral capsid (CA) core and targets its premature disassembly or destruction (*2*, *3*). Each primate species encodes a TRIM5α with a different antiviral specificity (*4*). For example, TRIM5α encoded by rhesus macaques renders them resistant to infection by HIV-1, but human TRIM5α affords no such protection (*5*). Indeed, the antiviral specificity of TRIM5α has rapidly evolved by dramatic episodes of positive selection during the past 30 million years of primate evolution (*6*). The branch leading to the human lineage shows one of the strongest signatures of positive selection (*6*), which suggests that at least one major pathogenic retroviral assailant has challenged the human lineage in the past 4 to 5 million years. Taken together, these findings suggest that TRIM5α evolution was shaped by a species-specific history of ancestral retroviral challenges. Although human TRIM5α has relatively poor activity against retroviruses compared with the gene from other primates, it potently blocks a γ-retrovirus N-MLV, which is related to PtERV1 (*7*, *8*). We therefore tested the hypothesis that TRIM5α may have protected early humans from invasion by PtERV1.

All copies of PtERV1 in the chimpanzee genome have been inactivated by accumulated detrimental mutations (*1*). However, the numerous proviral copies of PtERV1 present in the chimpanzee genome allow us to reconstruct the ancestral sequence of the *gag* gene of this ancient, extinct retrovirus in silico (see supporting online material). Analysis of the reconstructed PtERV1 ancestral sequence reveals about 50% identity with murine leukemia virus (MLV), and several characteristic conserved elements are intact (Fig. 1A). Phylogenetic analysis of chimpanzee and gorilla PtERV sequences shows that a single-source virus likely infected both chimpanzees and gorillas because viral sequences from both species form a monophyletic group (Fig. 1B).

We next used site-directed mutagenesis to reconstruct the ancestral p12 and CA coding regions (ignoring synonymous changes) starting from one chimpanzee PtERV1 provirus cloned from the genome. We focused on CA because it is the functional target of TRIM5α and included p12 because of functional interactions that exist between p12 and CA in other γ-retroviruses (*9*). Because TRIM5α interacts with the retroviral CA only in the multimeric structure characteristic of mature retroviral particles (*10*), we generated the PtERV1 capsid core in the context of an infectious virus capable of only a single round of infection. This was achieved by constructing a chimeric virus between PtERV1 and MLV that encodes a *gag/pol* gene expressing the reconstructed p12 and CA proteins of PtERV1 with the remainder of the viral structural proteins and enzymes of MLV (Fig. 1C). Our MLV/PtERV1 chimeric virus was indeed infectious (Fig. 1D), which demonstrates that regions of a 3- to 4-million-year-old primate endogenous retrovirus can be successfully resurrected.

We tested human TRIM5α restriction of PtERV1 by infecting cells that express an exogenous copy of human TRIM5α. A much younger human endogenous retrovirus, HERV-K, was also recently resurrected (*11*, *12*) but was not restricted by human TRIM5α (*12*). In contrast, expression of human TRIM5α in a heterologous cell type resulted in a dramatic reduction of infectivity of the MLV/PtERV1 chimera by a factor of more than 100 compared with cells that do not express TRIM5α (Fig. 2A). These data indicate that humans possess an intrinsic immunity gene capable of effectively neutralizing an extinct retrovirus that never successfully fixed into the human genome.

Specificity of TRIM5α for a particular retroviral capsid is largely determined by amino acids within the C-terminal B30.2 domain. Within this

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capsid-interaction domain, a particular "patch" contains amino acids under positive selection that contain the dominant determinants of specificity (*6*, *13*, *14*). For example, the amino acid at position 332 within this patch is a critical determinant of HIV-1 restriction (*13*). Humans and chimpanzees encode an arginine (R) residue at position 332, whereas the hominoid ancestral residue at this position is a glutamine (Q). Reversing this

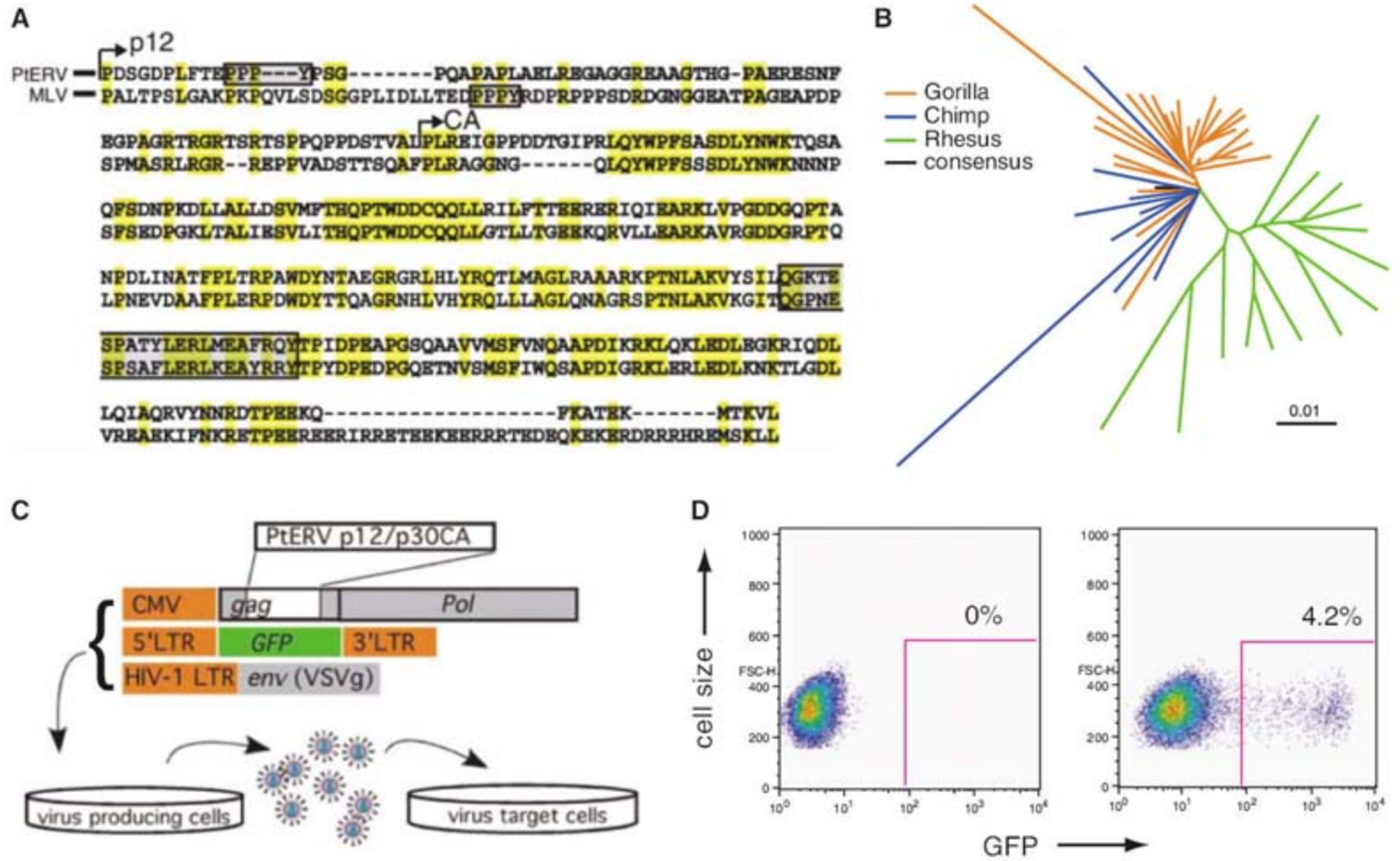

Fig. 1. Reconstruction of PtERV1 p12 and CA and infectivity of PtERV1/MLV chimeric virus. (**A**) An alignment of the p12 and CA sequences of the consensus PtERV1 from chimpanzee (top line) and MLV (bottom line) is shown. A late domain (PPPY) characteristic for γ retroviruses is in p12 and the Major Homology Region (MHR) conserved in all retroviruses is present in CA are indicated by shaded boxes. (**B**) An unrooted phylogenetic NJ tree was generated with nucleotide sequences of PtERV1 (MA through PRO) from chimpanzee, rhesus, and gorilla genomes. Branches are color coded: gorilla, orange; chimpanzee, blue; and rhesus, green. Fifteen amino acid changes were introduced into one extant copy of PtERV1 p12-CA from chimpanzees to generate the "ancestral" clone (fig. S1). The derived consensus sequence from chimpanzee is denoted by a black branch. The nucleotide consensus is slightly displaced from the node of the monophyletic clade containing chimp and gorilla sequences because synonymous changes were not introduced during the reconstruction process. (**C**) Schematic representation of the chimeric virus between MLV and PtERV1 is shown. The Gag-Pol expression vector used to make virus contains the MA, NC, and the entire POL coding sequence of MLV and the p12 and CA from PtERV1. Protease cleavage sites between MLV MA and PtERV1 p12, as well as between PtERV1 CA and MLV NC, were generated to match cleavage sites recognized by MLV protease. (**D**) Green fluorescent protein (GFP)–encoding virus was used to infect Crandell-Rees feline kidney (CRFK) cells with PtERV1 chimeric virions. Fluorescence-activated cell sorting (FACS) plots of virus-challenged cells are shown. The *y* axis indicates cell size, and the *x* axis denotes GFP positivity, which is an indicator of transduction. The initial PtERV1 clone used to reconstruct this consensus was tested in the chimeric context and was noninfectious (left). After 15 mutations were introduced to reconstruct the consensus p12 and CA sequence, infectious virus particles were generated (right).

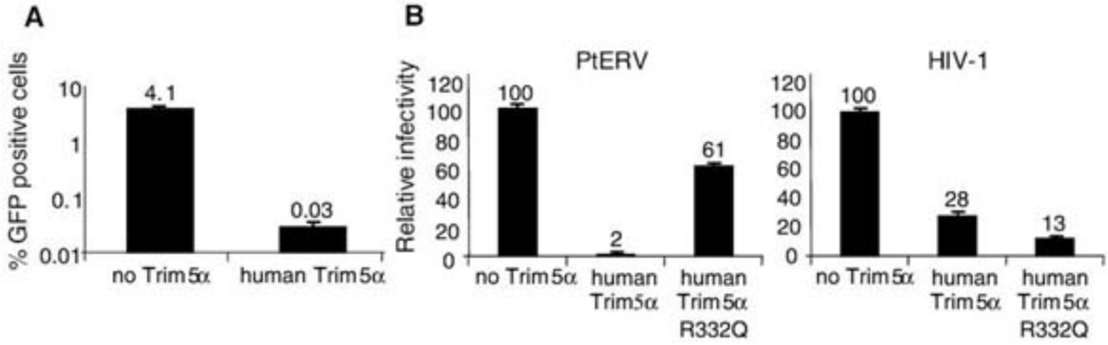

Fig. 2. Human TRIM5α restricts PtERV1 and restriction maps to a single residue within the interaction domain. (**A**) TRIM5α cloned from human cDNA was stably transduced into feline CRFK cells. These human TRIM5α-expressing cells along with CRFK control cells were infected with a GFP-encoding PtERV1 in a single-round infection assay. (**B**) Human TRIM5α with an R332Q mutation was generated and stably expressed in CRFK cells. These cells, along with cells expressing wild-type human TRIM5α, were used as targets for GFP-encoding PtERV1 or HIV-1. Infections were done in triplicate.

change (R332Q) had moderate effects on the ability of human TRIM5α to restrict MLV variants (fig. S2). Notably, changing the arginine to the ancestral glutamine abolished the ability of human TRIM5α to efficiently restrict PtERV1 infectivity (Fig. 2B). Unexpectedly, the R332Q mutation had the opposite effect on HIV-1, improving the ability of human TRIM5α to restrict this virus (*15*) (Fig. 2B). Thus, the R332Q mutation in human TRIM5α reveals a trade-off in TRIM5α's ability to restrict two retroviruses; a mutation that abolished restriction for PtERV1 results in a gain of restriction to other viruses such as HIV-1.

We tested the idea that restriction of PtERV1 and HIV-1 by TRIM5α is mutually exclusive by cloning and testing a panel of TRIM5α genes from other primates (Fig. 3, A and B). Indeed, we found no case where a primate encodes a TRIM5α capable of efficient restriction of both PtERV1 and HIV-1 (Fig. 3A). This is especially notable in TRIM5α from Old World monkeys where, for example, TRIM5α from baboons and African green monkeys have fifty-fold restriction of HIV-1 but negligible restriction of PtERV1. In contrast, that from sooty mangabeys has a fifty-fold restriction of PtERV1 but negligible restriction of HIV-1 (Fig. 3A). The same effect, although less extreme, was seen in the hominoid TRIM5α alleles, except in orangutan and gibbon, which are poor restrictors of both viruses. Thus, during primate evolution, a gain of restriction for one virus appears to have coincided with a loss of restriction against another virus.

Although we cannot rule out the possibility that PtERV1 never infected human ancestors for other reasons (SOM Text, note 1), our data do suggest the possibility that TRIM5α was fixed in human populations because of its ability to confer protection against PtERV1 (Figs. 2 and 3) and that modern humans have descended from ancestors who resisted infection. Indeed, we know that there is very little diversity in the human population today in the part of TRIM5α that determines antiviral specificity (*6*, *16*, *17*). However, we find that chimpanzee TRIM5α is also capable of restricting PtERV1 and encodes an R332 (Fig. 3), yet chimpanzees contain multiple copies of PtERV1 in their genome and humans do not. Moreover, we find that R332 is monomorphic in the TRIM5α allele in all four subspecies of chimpanzees and in bonobos, which indicates that R332 is evolutionarily conserved through the chimpanzee radiation (in the past 1 to 2 million years). The most parsimonious explanation for the presence of R332 in humans and chimpanzees is that the mutation was fixed in our common ancestor, which presents a paradox because chimpanzee TRIM5α did not protect them against PtERV1. This suggests that TRIM5α alone does not determine retroviral invasion into the germline but that the combination of multiple retroviral restriction factors that are also rapidly evolving, such as the Apobec3 family (*18*), are necessary to explain ancient transmission events.

A second evolutionary scenario is that the human and chimp lineages independently fixed R332 after the species diverged. We know that the amino acid at position 332 has indeed changed independently in many different primate lineages, even among very closely related species (*6*, *19*). Moreover, at least two Old World monkey species harbor multiple alleles of TRIM5α that are polymorphic at position 332 and are likely maintained by balancing selection (*20*). Given the intensity of positive selection acting on this position, the less parsimonious explanation of independent fixation is not unlikely. In this scenario, infections in the chimpanzee lineage subsequent to PtERV1 could have driven the selection of R332.

Finally, our data show that resistance to PtERV1 comes at a cost because it reduces resistance to other retroviruses, such as HIV-1. Although retroviruses similar to HIV-1 are also found in chimpanzees and in gorillas (*21*, *22*), we do not yet know how the degree of antiviral activity conferred by TRIM5α relates to resistance or sensitivity to infection at the organismal level. Nonetheless, our analyses demonstrate that selection for resistance to one potential retroviral pathogen could render a host defense gene less capable of inhibiting another virus. We speculate that fixation of a mutation in human TRIM5α, which could have protected early humans from viruses such as PtERV1 in our distant evolutionary past, may be at least partially responsible for our currently poor resistance to HIV-1.

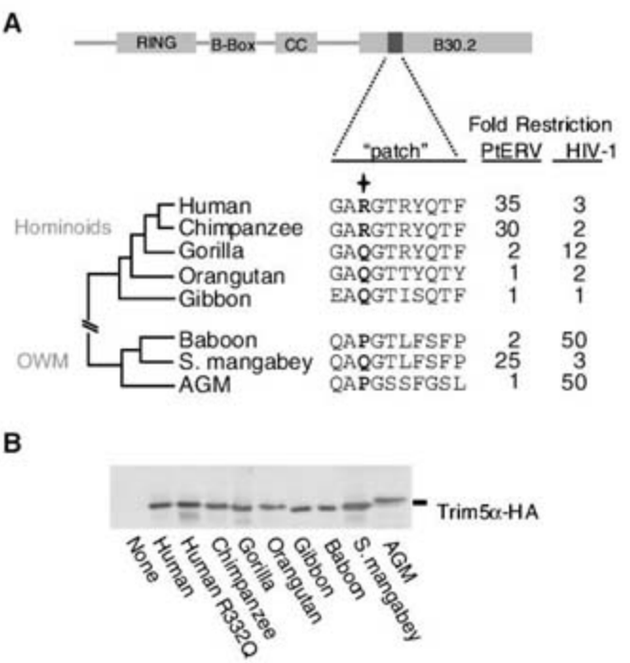

Fig. 3. Mutually exclusive restriction of PtERV1 and HIV-1 by primate TRIM5αs. (**A**) Primate TRIM5α genes were cloned, and hemagglutinin (HA)–tagged cDNAs were stably expressed in CRFK cells. Each stable cell line was assayed for restriction by PtERV1 or HIV-1 in a single-round infection assay using GFP-encoding viruses. Shown is a schematic representation of TRIM5α protein domains. The sequence of amino acids ("patch") within the B30.2 interaction domain that partially determines retroviral specificity is indicated for each primate tested, along with a cladogram of accepted primate phylogeny including the hominoid clade (top) and selected Old World monkeys (bottom). The asterisk indicates amino acid 332. Restriction of PtERV1 or HIV-1 is expressed as average fold restriction of three independent infections compared with a no-TRIM5α control. SDs were negligible. (**B**) Expression of each primate TRIM5α was similar, as detected by Western Blot analysis for the N-terminal HA-epitope tag.

References and Notes

1. C. T. Yohn *et al.*, *PLoS Biol.* **3**, e110 (2005).
2. M. Stremlau *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 5514 (2006).
3. X. Wu, J. L. Anderson, E. M. Campbell, A. M. Joseph, T. J. Hope, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 7465 (2006).
4. B. Song *et al.*, *J. Virol.* **79**, 3930 (2005).
5. M. Stremlau *et al.*, *Nature* **427**, 848 (2004).
6. S. L. Sawyer, L. I. Wu, M. Emerman, H. S. Malik, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2832 (2005).
7. M. J. Perron *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 11827 (2004).
8. T. Hatziioannou, D. Perez-Caballero, A. Yang, S. Cowan, P. D. Bieniasz, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 10774 (2004).
9. S. K. Lee, K. Nagashima, W. S. Hu, *J. Virol.* **79**, 4159 (2005).
10. C. C. Mische *et al.*, *J. Virol.* **79**, 14446 (2005).
11. M. Dewannieux *et al.*, *Genome Res.* **16**, 1548 (2006).
12. Y. N. Lee, P. D. Bieniasz, *PLoS Pathog.* **3**, e10 (2007).
13. M. W. Yap, S. Nisole, J. P. Stoye, *Curr. Biol.* **15**, 73 (2005).
14. M. Stremlau, M. Perron, S. Welikala, J. Sodroski, *J. Virol.* **79**, 3139 (2005).
15. Y. Li, X. Li, M. Stremlau, M. Lee, J. Sodroski, *J. Virol.* **80**, 6738 (2006).
16. H. Javanbakht *et al.*, *Virology* **354**, 15 (2006).
17. S. L. Sawyer, L. I. Wu, J. M. Akey, M. Emerman, H. S. Malik, *Curr. Biol.* **16**, 95 (2006).
18. S. L. Sawyer, M. Emerman, H. S. Malik, *PLoS Biol.* **2**, E275 (2004).
19. H. L. Liu *et al.*, *Gene* **362**, 109 (2005).
20. R. M. Newman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19134 (2006).
21. F. Van Heuverswyn *et al.*, *Nature* **444**, 164 (2006).
22. B. F. Keele *et al.*, *Science* **313**, 523 (2006).
23. We thank M. Rogel for initiating the PtERV1 reconstruction; E. Eichler and B. Hahn for chimpanzee DNAs; the Fred Hutchinson Cancer Research Center flow cytometry lab; and E. Eichler, M. OhAinle, S. Sawyer, Y. Voronin, and M. Yamashita for comments on the manuscript. This work was supported by NIH grants (M.E.) and a Searle Scholar Award (H.S.M.). S.M.K. was supported by an NSF graduate fellowship.

Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5832/1756/DC1
Materials and Methods
SOM Text
Figs. S1 and S2
References

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An Antifungal Agent Inhibits an Aminoacyl-tRNA Synthetase by Trapping tRNA in the Editing Site

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Aminoacyl–transfer RNA (tRNA) synthetases, which catalyze the attachment of the correct amino acid to its corresponding tRNA during translation of the genetic code, are proven antimicrobial drug targets. We show that the broad-spectrum antifungal 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2690), in development for the treatment of onychomycosis, inhibits yeast cytoplasmic leucyl-tRNA synthetase by formation of a stable $tRNA^{Leu}$-AN2690 adduct in the editing site of the enzyme. Adduct formation is mediated through the boron atom of AN2690 and the 2′- and 3′-oxygen atoms of tRNA's 3′-terminal adenosine. The trapping of enzyme-bound $tRNA^{Leu}$ in the editing site prevents catalytic turnover, thus inhibiting synthesis of leucyl-$tRNA^{Leu}$ and consequentially blocking protein synthesis. This result establishes the editing site as a bona fide target for aminoacyl-tRNA synthetase inhibitors.

Aminoacyl-tRNA synthetases (AARSs) perform a pivotal role in translating the genetic code by catalyzing the attachment of the correct amino acid to its cognate tRNA (*1*). The aminoacylation reaction occurs in two steps: the formation of an enzyme-bound aminoacyl-adenylate, followed by transfer of this activated amino acid to either the 2′- or 3′-hydroxy group on the 3′-terminal adenosine of tRNA. The accuracy of the tRNA aminoacylation reaction is critical to ensuring the fidelity of the genetic code (*2*). To achieve this accuracy, many AARS enzymes possess a proofreading (editing) mechanism that hydrolyzes tRNAs aminoacylated with the incorrect amino acid (*3*). Leucyl-tRNA synthetase (LeuRS) is a proofreading AARS, which possesses distinct synthetic (aminoacylation) and editing active sites separated by more than 30 Å (*4*, *5*). We show that 5-fluoro-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (AN2690) inhibits LeuRS by trapping $tRNA^{Leu}$ in the editing active site.

AN2690 is a member of a new class of broad-spectrum antifungals (table S1), the benzoxaboroles, which have an unusual chemical attribute: a boron atom (*6*). We isolated spontaneous and ethyl-methanesulfonate (EMS)–induced AN2690-resistant mutants in the yeast *Saccharomyces cerevisiae* (*7*). These genetically dominant mutants were 32- to 512-fold more resistant to AN2690 than the parental *S. cerevisiae* strain (table S2), and their resistance mutations were found to lie in the *CDC60* gene, which encodes the cytoplasmic LeuRS (Cdc60p). Furthermore, all AN2690-resistant mutations mapped to the editing domain (Fig. 1A and table S2) and all but two, $Cys^{326} \rightarrow Arg^{326}$ (C326R) and $Cys^{326} \rightarrow Phe^{326}$ (C326F) (*8*), to the two highly conserved regions that form the editing active site of LeuRS (*9*). Four mutations lie in the threonine-rich region, a locus known in bacterial LeuRS homologs to be involved in binding and hydrolyzing mischarged tRNAs (*9*–*12*). Seven of the nine mutants exhibited an editing defect based on their sensitivity to the structurally related noncognate amino acid norvaline (fig. S1). These results suggest that the editing pocket of Cdc60p is the binding site for AN2690.

To delineate its mode of action, we investigated the effect of AN2690 on the ability of LeuRS to hydrolyze mischarged $tRNA^{Leu}$. Addition of AN2690 to the posttransfer editing assay inhibited the hydrolysis of Ile-$tRNA^{Leu}$ in a dose-dependent manner (Fig. 1B). In addition, we found that AN2690 inhibited tRNA aminoacylation (fig. S2A), and, as would be expected for a LeuRS inhibitor, it blocked protein synthesis in vivo (fig. S2B). Initial aminoacylation experiments also revealed that AN2690 required the presence of tRNA for effective inhibition of aminoacylation activity. Kinetic analysis of aminoacylation inhibition showed that AN2690 acted as a noncompetitive inhibitor with respect to both adenosine triphosphate (ATP) and leucine (fig. S3, A and B). Analysis of the noncompetitive nature of AN2690 revealed that the inhibition constant (K_i) decreased on increasing AN2690's incubation time with tRNA and Cdc60p, before initiating the aminoacylation reaction with ATP. When enzyme and tRNA were incubated with AN2690 for 2 min, the K_i was 31.4 ± 2.8 (SEM) μM, whereas after a 20-min incubation the K_i decreased to 1.85 ± 0.1 μM (fig. S3). To better understand this process, we measured inhibition of aminoacylation as a function of incubation time and AN2690 concentration (Fig. 2A). We found a direct linear relationship between the observed rates of inactivation (k_{obs}) and AN2690 concentrations, with no apparent plateau even at the highest concentration tested (fig. S4). From these data, we deduced a rate of inactivation of the enzyme ($k_{inactivation}$) of 0.66 ± 0.10 min^{-1}

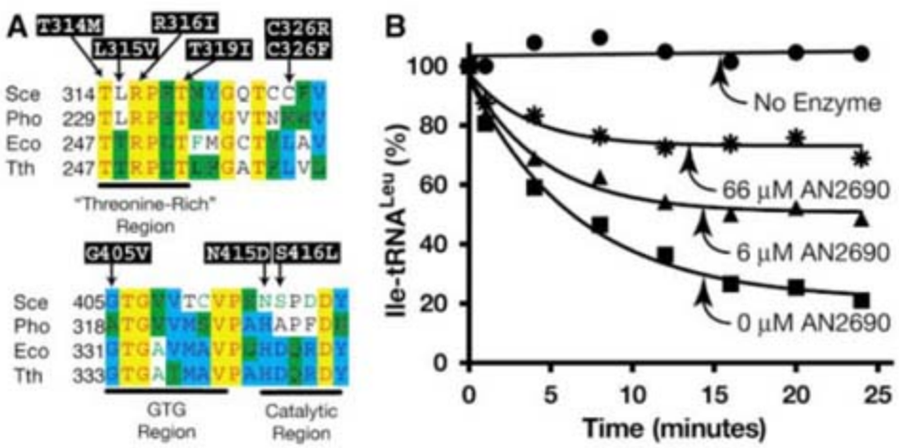

Fig. 1. (**A**) *S. cerevisiae* AN2690 resistance mutations in the editing active site of Cdc60p (*8*). Alignment of the conserved regions of the LeuRS editing domains from *S. cerevisiae* (Sce) from CAA97865, *Pyrococcus horikoshii* (Pho) from O58698, *Escherichia coli* (Eco) from AAC73743, and *T. thermophilus* (Tth) from BAD69984. The amino acid substitutions that confer resistance in *S. cerevisiae* to AN2690 are in black (table S2). (**B**) AN2690 inhibits posttransfer editing. Deacylation of total brewer's yeast tRNA mischarged with isoleucine, no enzyme control (circles), enzyme control (squares), Cdc60p treated with 6 μM AN2690 (triangles), and enzyme treated with 66 μM AN2690 (asterisks). All reactions were performed in triplicate, and the mean values were plotted. The SD for each point was less than 4%.

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(fig. S5). Measurement of the rate of recovery demonstrated that AN2690's inactivation of Cdc60p was reversible, albeit with a very slow reactivation rate of $1.64 \times 10^{-4} \pm 0.15 \times 10^{-4}$ min^{-1} (Fig. 2B), which corresponds to a half-life of 424 min. These kinetic properties are consistent with AN2690 being characterized as a slow-tight-binding inhibitor.

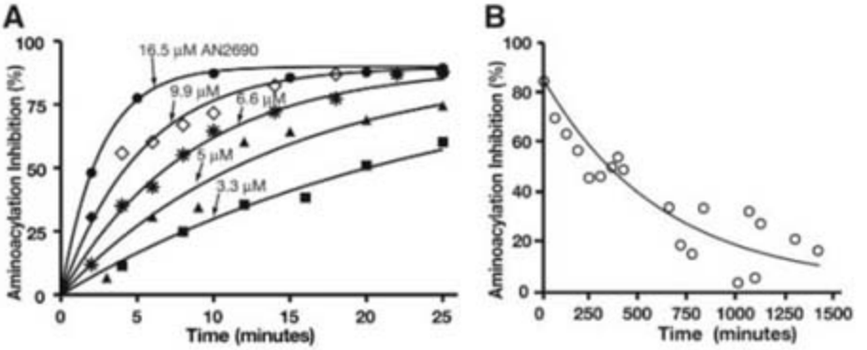


Fig. 2. (**A**) The rate of Cdc60p inactivation by AN2690. The percentage of inhibition from different concentrations [3.3 μM (squares), 5 μM (triangles), 6.6 μM (asterisk), 9.9 μM (open diamonds), and 16.5 μM (circles)] of AN2690 were plotted versus the time of incubation. All reactions were performed in triplicate, and the mean values were plotted. (**B**) The recovery of Cdc60p activity from AN2690 inhibition. All reactions were performed in triplicate, and the mean values were plotted. The data were fitted to a first-order exponential decay.

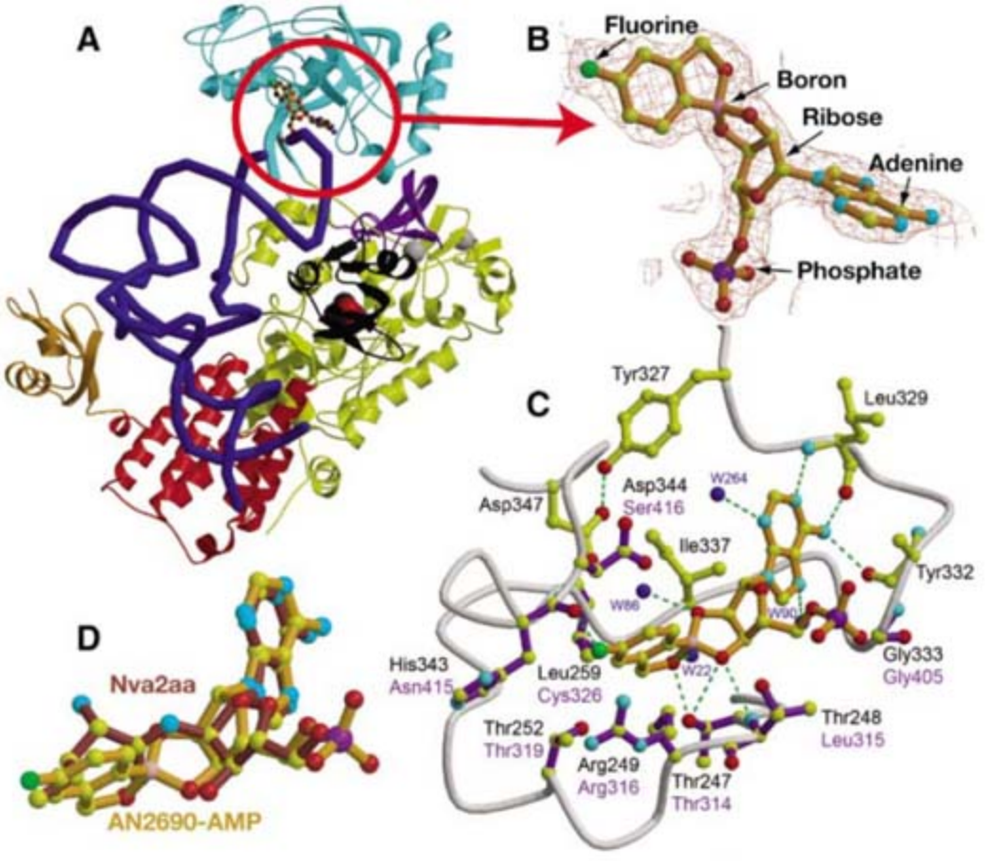


Fig. 3. AN2690 forms an adduct with the terminal adenosine (A76) of $tRNA^{Leu}$ in the editing active site of LeuRS. (**A**) Overall structure of the complex of *T. thermophilus* LeuRS with $tRNA^{Leu}$ and AN2690, showing the adenosine-AN2690 adduct (ball-and-stick model, ringed in red) in the editing site and leucine (space-filling model) in the synthetic site. The editing domain is cyan; the catalytic domain, yellow; Zn-1 domain, purple; the leucyl-specific insertion domain, black; the anticodon-binding domain, red; the C-terminal domain, gold; zinc atoms, gray spheres; and tRNA, blue tube. (**B**) Unbiased difference map (1.85 Å resolution) for the AMP-AN2690 adduct in the editing site. (**C**) Diagram showing water molecules (dark blue spheres) and hydrogen bonds (green dotted lines) between editing site residues of LeuRS and the AMP-AN2690 adduct (orange). Amino acid residues that are mutated in the *S. cerevisiae* AN2690-resistant mutants are labeled and colored in purple (table S2). The atoms are colored accordingly: boron, mauve; fluorine, green; oxygen, red; nitrogen, light blue; carbon, yellow; and phosphate, purple. (**D**) Superposition of bound posttransfer editing substrate analog (Nva2AA, brown) (*9*) and the AMP-AN2690 adduct (orange) obtained after superposing the Cα positions of the editing domain of each complex.

Equilibrium dialysis demonstrated that adenosine-containing ribonucleotides were essential for measurable binding of AN2690 to Cdc60p (fig. S6A). To determine more precisely the mode of action of AN2690, we obtained a 3.5 Å crystallographic structure of *Thermus thermophilus* LeuRS complexed with $tRNA^{Leu}$ and AN2690 (Fig. 3A). Significant positive difference density found in the editing site (fig. S7) was interpreted as a tRNA-AN2690 adduct with the boron from the oxaborole ring bound to the 2′,3′-hydroxy groups on the 3′-terminal adenosine (A76). A 1.85 Å resolution structure of an adenosine monophosphate (AMP)–AN2690 adduct bound in the editing site of LeuRS (Fig. 3B) confirmed this tetrahedral spiroborate structure, whose configuration is stabilized by two hydrogen bonds to the conserved threonine-rich peptide and a water molecule (Fig. 3C). These results show that AMP can act as a surrogate for the 3′-terminal adenosine of $tRNA^{Leu}$, as in our direct binding assay, and that AN2690 occupies the noncognate amino acid–binding pocket in the editing site. The adenosine and the 2′-hydroxy group of the AMP-AN2690 adduct can be exactly overlaid on the analogous groups of a posttransfer editing substrate analog, 2′-(L-norvalyl) amino-2′-deoxyadenosine (Nva2aa), bound in the editing site (*9*). However, the planar benzoxaborole only partially overlaps with the noncognate amino acid (Fig. 3D). In particular, AN2690 has no equivalent of the amino acid amino group, and the absence of any associated strong interactions with the enzyme probably explains why the compound has very low affinity for the editing site in the absence of AMP or tRNA.

Because benzoxaboroles can bind to the *cis*-diols of sugars (*13*, *14*), we tested the requirement for adenosine's 2′- and 3′-hydroxy groups in AN2690 binding to Cdc60p and found that both hydroxy groups were required (fig. S6C). This implies that AN2690 can only form an

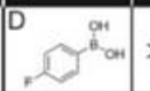
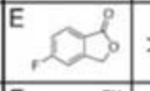
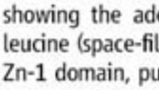
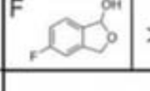
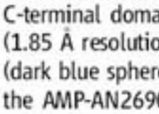
Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
AN2690	2.1	D 	>100
A 	96	E 	>100
B 	>100	F 	>100
C 	>100		

Fig. 4. Boron and the oxaborole ring are required for inhibition of aminoacylation. All reactions were performed in triplicate, and the mean values were used to determine a median inhibitory concentration (IC_{50}) with Prism 4 (*17*).

CELL SIGNALING:

IN VIVO VERITAS

Innovative and increasingly sophisticated technologies are allowing scientists to visualize signaling pathways and cellular interactions with finer detail than ever before. New types of fluorescent tags and novel chemistries, in conjunction with technological improvements in microscopes, are driving research forward at speed. Pausing for a moment, this article looks under the hood at the science behind the technology—from quantum dots to microendoscopy, and beyond.

By Jeffrey M. Perkel

Imagine trying to understand the rules of football by taking a single photograph of a particular game at a specific moment in time, and comparing it with other photographs taken at other times, during different games. Because each game is unique, naturally, you would fail.

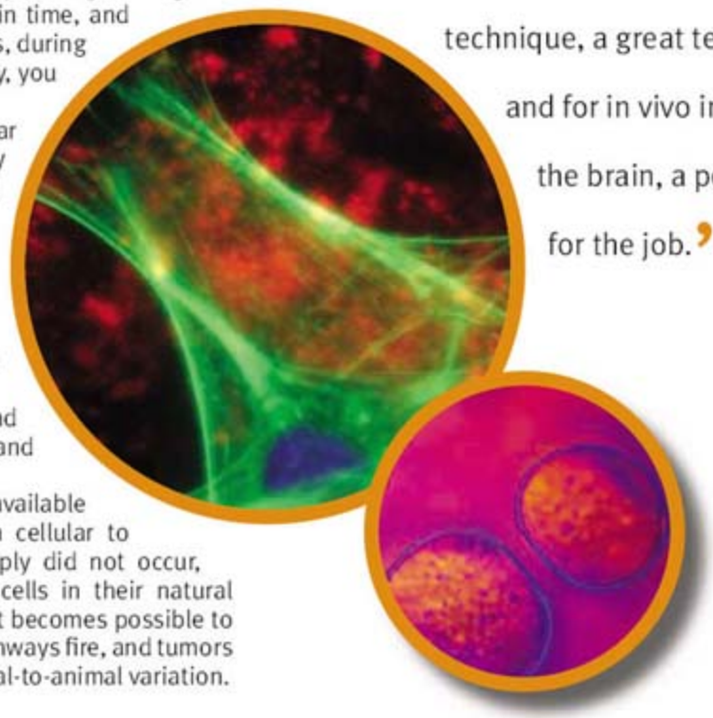
Yet according to Jeff Lichtman, professor of molecular and cellular biology at **Harvard University**, this is precisely the strategy most biologists take as they try to understand dynamic cellular events.

Consider cell shape, for instance. "If you see some object in different animals or different cells, the object has different shapes," he says. "That could mean that there are 20 different shapes, or it could mean that every object is constantly morphing. There's no way to know unless you watch the same object over time."

You have to sit in the bleachers for the whole game and study tapes of the play action, if you really want to understand the dynamics, he says.

That's where in vivo imaging comes in. Tools are now available to image cellular events in living animals at levels from cellular to organismal—events that either were inaccessible, or simply did not occur, on fixed slides and tissue culture dishes. By observing cells in their natural environments, in living animals rather than fixed on slides, it becomes possible to watch as neurons extend and retract, signal transduction pathways fire, and tumors invade new tissues—all without having to worry about animal-to-animal variation.

"Two-photon microscopy is a remarkable technique, a great technology, and for in vivo imaging of the brain, a perfect tool for the job."



Two Photons Better Than One

Lichtman's lab focuses on how neural connections are established during development. Early in development, individual muscle cells are enervated by many neurons. But over time, most of these nerve fibers retract, until just one remains.

To figure out how this happens, and which is the last neuron standing, Lichtman and his team have effectively taken to camping out in the molecular bleachers and watching as these events unfold.

The two teams in this case are neurons, some yellow, and some blue—the result of mating mice that express either of two fluorescent protein variants. These colored cells are tagged as with team jerseys, Lichtman explains, and he can scan the nerve-muscle interface in the exposed tissue of an anesthetized, immobilized animal until he finds a blue and a yellow axon in close proximity. Then, he says, "We look at the blue and yellow axon on one day, and look the next day, and keep doing that until one of the two inputs disappears."

Lichtman's lab records these processes using both confocal and two-photon imaging. Confocal imaging uses a laser to sweep back and forth across a sample, and a small pinhole to block light emanating from above or below the plane of focus. The result is a sharp image of a single, virtual slice of the specimen. The problem is that as in-focus light exits the sample, it can get deflected by other cells and cell components, and thus not reach the pinhole. Less and less emitted light is visualized as depth increases, so confocal really is effective only for near-surface imaging.

continued ›

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"Being able to go into the deep brain, where no microscope has ever been, period, and to make images with a resolution of even three micrometers is huge progress."

Two-photon microscopy eliminates the pinhole, relying instead on an optical trick to create virtual sections. Consider a fluorophore that normally fluoresces when stimulated by a single 400-nm photon. In two-photon microscopy, photons of light at half the energy and twice the wavelength—that is, at 800 nm—are directed in a cone-shaped beam toward the sample in intense bursts. These photons arrive so closely together that the fluorophore acts as if it has captured a single 400 nm photon, and so fluoresces.

Only fluorophores in the focal plane (at the tip of the cone) will respond in this way, because that is where the density of photons is greatest. Those outside the focal plane, which are unlikely to capture two photons in time, remain silent. "That means you can look through very thick tissue and only see fluorescence from the point of focus," Lichtman says. "It's a remarkable technique, a great technology, and for in vivo imaging of the brain, a perfect tool for the job."

Adding to two-photon's appeal is the system's use of near-infrared (NIR) laser excitation light (700–1,100 nm). Unlike shortwave illumination, NIR light passes through living tissues relatively unimpeded. As a result, the technique can be used to probe deep tissue, up to 600 microns or so. It also is less damaging than shorter wavelength light, and is thus compatible with longitudinal studies.

"There's an awful lot of brain processing on the surface of the cortex," says R. Clay Reid, professor of neurobiology at **Harvard Medical School**, "so there's a lot of in vivo neuroscience that one can do and be limited to 600 microns."

An electrophysiologist by training, Reid says he has "a new lease on life" thanks to two-photon microscopy and cell-permeable calcium indicators like Oregon Green, which he "spritzes" into the surface of the brain to light up neurons as they fire in response to different visual stimuli.

"Electrophysiology is the gold standard for understanding how things work," Reid says, "but electrophysiology is one cell at a time or, with multiple electrodes, a small fraction of a circuit." Now, "We can see and therefore record the activity of literally every single cell, and that's the exciting part. We are never going to understand the brain one cell at a time."

Going Deeper

All the major microscope manufacturers—**Nikon, Leica, Zeiss**, and **Olympus**—address two-photon imaging in one way or another, providing either complete systems or the specialized optics needed to upgrade existing microscopes.

Yet the technique's power is generally restricted to those cells within 600 to 700 microns of the tissue surface at best. "Most of the mouse brain is out of reach of a traditional two-photon microscope," says Mark Schnitzer, a **Stanford University** researcher who has developed techniques using microendoscopic objective lenses as small as 350 microns in diameter to bring both one- and two-photon imaging deeper into the brain.

Also delving deep into tissue is Olympus' IV100 intravital imaging system, which features slim MicroProbe "stick" lenses, 1.3- or 3.5-mm wide and mounted on a tilting scanhead, to enable deep tissue and internal organ laser-scanning imaging, at angles from +10 to +70 degrees, through a small incision.

Mauna Kea Technologies in Paris, France, has commercialized its own deep-body laser-scanning system, the Cellvision-LAB, distributed by Leica Microsystems. Instead of glass lenses, "the objective of the [Cellvizio-LAB] microscope is a bundle of tens of thousands of optical fibers," says Sacha Loiseau, Mauna Kea's president and chief executive officer. About the thickness of three human hairs, this objective can be threaded through the animal to image just about anywhere in the body.

Harvard's Reid, who used the Cellvizio to recapitulate his two-photon work in portions of the brain that otherwise were inaccessible, says the system offers greater depth, but lacks the resolution and versatility of regular two-photon imaging.

According to Loiseau, the resolution of the Cellvizio-LAB is 1.1 microns or so, about a fifth of the 0.2-micron theoretical resolution limit of traditional optical microscopes.

"It's a tradeoff," he says. "Being able to go into the deep brain, where no microscope has ever been, period, and to make images with a resolution of even three micrometers is huge progress."

The Wide-Angle View

Still other companies offer a loftier, whole-animal view.

Whole-animal optical imaging enables researchers to visualize light-emitting cells or processes (such as tumorigenesis) through the skin of living animals. One of the challenges, says William McLaughlin, director of research and development at **Molecular Imaging Systems, Carestream Health** (previously an Eastman Kodak company), is making an imaging chamber that is dark enough, and with sufficiently low autofluorescence, that it becomes possible to keep a camera shutter open for minutes at a time while providing a range of excitation light and without creating significant background artifacts.

Astronomy-grade, low-noise, cooled CCD cameras provided the solution to the other technical problem with whole-body imaging: having a camera with sufficiently low background to reliably detect the faint light signals from within animals.

Xenogen, now part of **Caliper Life Sciences**, has traditionally focused on whole-body bioluminescence imaging, though its newest entry in this area, the IVIS (in vivo imaging system) Spectrum, can also record fluorescent signals.

In fluorescence imaging, a fluorophore—whether continued ›

TO RED AND BEYOND

When it comes to in vivo imaging, you need a dark box, and a good probe. Lots of companies can provide the box; some are listed in the main text. Others include **Advanced Research Technologies'** eXplore Optix and **LI-COR Biosciences'** Odyssey.

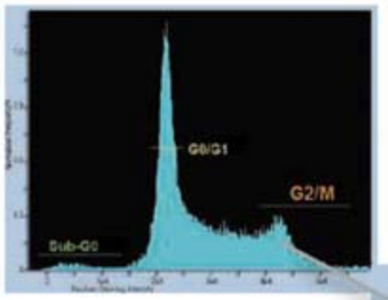
Thanks to the work of Roger Tsien at the **University of California at San Diego**, among others, researchers have literally dozens of fluorescent proteins to play with. No fluorescent protein yet reaches into the near infrared, but a few, such as Tsien's mPlum (emission maximum, 655 nm), and **Clontech's** Living Colors HcRed1 (618 nm), come close.

Several companies sell fluorescent imaging agents at or near the near-infrared, including LI-COR's IRDyes, **Invitrogen's** AlexaFluor-based SAIVI (small animal in vitro imaging) kits and QDot nanocrystal reagents, and **VisEn Medical's** VivoTags and NanoSPARKS.

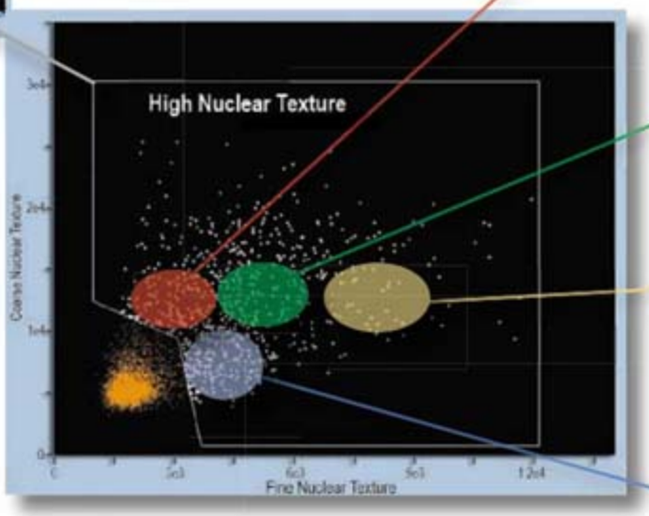
Some companies also offer probes specifically for detecting signaling events. VisEn's ProSense and MMPSense NIR sensors light up when cathepsin and matrix metalloproteinases are activated, respectively, for instance, while **Immunochemistry Technology's** SR-FLIVO probe marks apoptotic cells red.

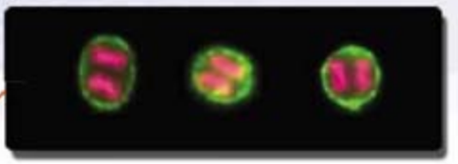
In flow cytometry, every dot tells a story . . .

The ImageStream® system



HLA60 cells were labeled with AF488 anti-HLA to reveal the cell membrane and with DRAQ5™ to stain the nucleus. Labeled cells were analyzed on the ImageStream system. The DNA content histogram (above) cleanly separates the major cell cycle subpopulations. G2/M cells were plotted (right) to display the high nuclear texture fraction, from which cells in the progressive stages of mitosis were identified and highlighted on the plot. Composite cell images (far right) show HLA and nuclear staining.

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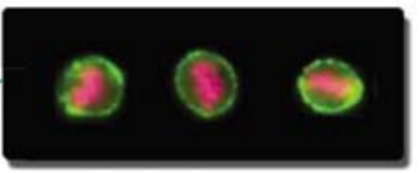
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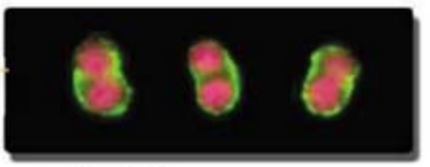
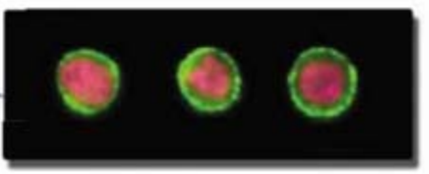

Telophase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an organic dye, quantum dot, or fluorescent protein—is irradiated with light of a specific wavelength, inducing it to emit light at a different wavelength, that is, to fluoresce. Bioluminescence imaging, in contrast, requires no excitation; the emitted light comes instead from the enzymatic turnover of exogenously introduced substrate molecules like luciferin.

Where luminescence is basically monochromatic (though Promega, for instance, does offer two luciferase variants that differ in their substrate requirements and emission spectra), there exists a veritable palette of fluorescent proteins and fluorophores to enable multiplexed detection of different proteins or events simultaneously. Oftentimes, these colors can appear identical. That's the problem **Cambridge Research & Instrumentation** (CRI) addresses with its fluorescence-based Maestro in vivo imaging system.

Suppose you shine a red light and a green light on a wall, says James Mansfield, CRI's director of multispectral imaging systems. "Where you only see red, it's red, where you only see green, it's green, and where they overlap they would appear yellow."

That yellow is different, however, from the color you would see if you also shined a yellow laser on the wall. "Both appear yellow to us, when spectrally they are very different."

Multispectral imaging allows researchers to distinguish such differences, which become important biologically when, say, trying to distinguish fluorescein (which glows green) from green fluorescent protein, or more important, from the tissue's inherent autofluorescence, long the bugaboo of in vivo fluorescence imaging.

Carestream Health brings yet another imaging modality to the table: X-ray.

According to Shahram Hejazi, president of Molecular Imaging Systems, Carestream Health, X-ray imaging provides anatomic landmarks so that the precise source of optical signals can be identified. "When you capture an optical image from inside an animal, there is no anatomical image in the background. You see a blip on a dark background, so you have no idea where it comes from."

By superimposing a high-resolution X-ray on that image, however, "you can see that the 'blip' is on the third joint in the finger, or between two vertebrae." The company's newest product is the Kodak In-Vivo Multispectral Imaging System FX, which combines optical imaging with spectral unmixing, radioisotopic imaging, and digital X-ray imaging in a single instrument.

Despite their differences, many whole-body systems image three-dimensional animals in two dimensions, like paper on a photocopier. But not all do. The Pan-A-See-Ya imaging system from **Lightools Research** simultaneously images the top, left, and right sides of an animal under identical illumination and without moving the animal, while VisEn Medical's Fluorescence Molecular Tomography system uses a raster-scanning excitation source to capture and render the animal's fluorescence signal in three dimensions. Xenogen also offers a 3D version of its IVIS instrument, which reconstructs its image from eight individual bioluminescence or fluorescence frames.

Imaging Signaling Events In Vivo

According to Sam Gambhir, director of the Molecular Imaging Program at Stanford University, what distinguishes optical imaging from traditional medical imaging modalities like X-ray, PET (positron emission tomography), MRI (magnetic resonance imaging), and CT (computed tomography) is merely that part of the physical spectrum the system is tuned to hear.

"When you have an X-ray, they shoot radiation through you to see how much comes out the other side," Gambhir says. "So an X-ray is a map of density. That says nothing about what's going on in the cells in your lungs or bones. To do that you need a technology that lets you listen in on molecular events."

Gambhir has spent years developing sensors targeting such molecular events as protein-protein interactions, phosphorylation, and protein folding.

"We have a toolbox that lets you go from the surface of the cell, to pathways, into the nucleus, and we can monitor all of these in intact cells while the cells are within living subjects," he says.

Signaling sensors cannot rely on antibodies, Gambhir says, because there is no easy way to get antibodies into living cells. Instead, he relies on signaling event–mediated activation of otherwise silent biosensors. In one example, a functional, light-emitting protein is split in two, rendering it nonfunctional. "If those segments are fused to the two proteins you are interested in, the proteins are brought together and you reconstitute their ability to make light," a procedure known as protein-assisted complementation, or PAC.

Ryohei Yasuda, assistant professor of neurobiology at **Duke University Medical Center** in Durham, North Carolina, uses another approach, called two-photon fluorescence lifetime imaging microscopy, to detect signaling events as synaptic connections change in thick, living tissue sections.

Yasuda detects interactions between the signaling protein Ras and its interaction partners by coupling the molecules to different fluorescent proteins and using fluorescence lifetime measurements to monitor changes in fluorescence resonance energy transfer between them. Though he has not yet migrated this technique into living animals, Yasuda says he sees "no real barrier" to doing so.

"All the techniques to do in vivo signaling imaging already exist," he says, even if they mostly have only been used in vitro.

The resulting data will surely provoke reevaluation of old assumptions, as Jan Schnitzer, Scientific Director of the **Sidney Kimmel Cancer Center** in San Diego, can attest.

Using a battery of in vivo and ex vivo techniques, Schnitzer and his team reported recently that caveolae—tiny membrane invaginations that play a role in endocytosis and macromolecular trafficking—are far more efficient at moving molecules across endothelia than previously recognized. It's an observation that could not have been made in cell culture, he says, and one that could transform the way drugs and imaging agents are delivered to patients.

"That's why it's important to go in vivo," he says. "In vivo veritas, and in medical technology, that's the only thing that matters."

Jeffrey Perkel is a freelance science writer based in Pocatello, Idaho.

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Informal enquiries to christine.struthers@ed.ac.uk

Further information about the Centre can be found at www.wcb.ed.ac.uk Further information about Wellcome Trust Career Development Fellowships can be found at www.wellcome.ac.uk/node2129.html

Applications, containing a CV (including names of three academic referees) and a brief research outline/proposal, should be sent to Professor Adrian Bird (Director), Wellcome Trust Centre for Cell Biology, University of Edinburgh, Michael Swann Building, The King's Buildings, Edinburgh EH9 3JR, UK. Applications may also be sent as Word (.doc) or PDF files by email to Professor Bird's secretary, email: christine.struthers@ed.ac.uk You should ask your referees to send letters of reference by the deadline. The deadline for applications is 15 August 2007. Interviews for short-listed candidates are likely to be held in late September or early October 2007.

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This is a new Chair at the Institute of Nuclear Medicine (INM) of UCL (University College London), established for the development of a molecular imaging programme based on radiolabelled ligands. A novel initiative aims to develop interfaculty links between Medicine and Chemical Biology at UCL in a programme of translational research. The Chair will be housed within the Centre for Chemical Biology and will develop technologies for the investigation of the phenotype expression of disease, from the laboratory to man, using advanced PETCT and SPETCT imaging technologies.

Significant opportunities have also arisen with the successful award of a Comprehensive Biomedical Research Centre arising from a collaborative partnership between University College London Hospitals (UCLH) and UCL.

The Chair will conceptually lead and develop the infrastructure and a team for the above programme, spanning the areas of oncology, cardiovascular and neurological applications. The ideal candidate will have an international reputation in radiopharmaceutical research, spanning the area from basic science to clinical applications, a proven record in obtaining grant support, and experience and or potential in running a multidisciplinary team. Salary will be negotiable on the professorial scale.

Informal enquiries may be made to Professor Peter Ell, Director, Institute of Nuclear Medicine, tel: +44 (0)207 631 1066, email: peter.ell@uclh.nhs.uk, or Professor Stephen Caddick, Professor of Organic Chemistry and Chemical Biology, Department of Chemistry, tel: +44 (0)207 679 4694, email: s.caddick@ucl.ac.uk. Further particulars and application details for the post can be obtained from Professor Ell.

We particularly welcome female applicants and those from an ethnic minority, as they are currently under-represented within UCL at this level.

Closing date: 31st August 2007.

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Informal enquiries can be made to the Head of School, Professor John Harwood (Harwood@cardiff.ac.uk), to the Joint Heads of Research, Professor Alan Clarke (ClarkeAR@cardiff.ac.uk) and Professor Kevin Fox (FoxKD@cardiff.ac.uk) or to the Head of Teaching, Professor Bernard Moxham (Moxham@cardiff.ac.uk).

Please include full details of publications and research grant income (as relevant) and an indication of your research plans. A covering letter of application, setting out personal career aspirations and the post currently held by the applicant must be attached.

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Europe's Wild West

The UK and Ireland are both reaching for the benefits of a knowledge economy. The UK leads Europe's biotechnology scene, and Ireland's rapid economic trajectory through the 1990s has radically altered the research landscape thanks to large injections of cash. International companies' search for research talent has intensified. Many have set up operations in the UK and Ireland where they tap into expertise from world-class universities, as well as opening their arms to researchers moving on from the competitive academic sector. **By Helen Carmichael**


Cian Lynch

"It feels as though this is the Wild West and there's a massive land grab of new ideas."

There's no question that the UK fosters world-class science. The government's 2004 10-year Science and Innovation Investment Framework set out a long-term vision for science and innovation, together with the ambition that public and private investment in R&D should reach 2.5 percent of GDP by 2014. Investment in infrastructure and funding for Ph.D.s is yielding results, although beyond Ph.D. level, obtaining funding and carving out a career still require determination.

Staying at the Top

Almost one in five of the world's leading 100 medicines was developed in the UK, which has the largest biotechnology sector in Europe. Major pharmaceutical companies continue to invest in infrastructure and to develop links with the academic and biotech communities, making the UK an attractive location for biomedical researchers to test their mettle.

Regional bioscience initiatives abound. In Scotland, for example, six universities will invest £77.4 million pooling their research excellence in the new Scottish Universities Life Sciences Alliance (SULSA). Eighteen new research posts and 24 support posts will be created at the universities of Aberdeen, Dundee, Edinburgh, Glasgow, St. Andrews, and Strathclyde.

Retaining Talent, Seeding Discovery

Cian Lynch is beginning postdoctoral research at the P53 Cancer Research Laboratory at the University of York, and has certainly embraced the frontier spirit in UK biomedical research. "It feels as though this is the Wild West and there's a massive land grab of new ideas." Lynch hails from Ireland, where he studied biochemistry at University College Cork. "Everyone was advising me to go to the UK," he says. There were fewer opportunities in Ireland at the time, and he started out in Cambridge, UK, as a research assistant but was soon offered a Ph.D. opportunity at York.

Toward the end of his Ph.D., Lynch decided that industry was not for him. "I started looking at America: that's where people sometimes earn their stripes before they come back here." Although he was tempted by a project in California, Lynch accepted an offer to stay in his lab at York, an indication that UK projects increasingly rival the allure of US opportunities.

In addition to government and industry funding, charities contribute significantly to UK research. The Wellcome Trust supports basic human and animal health research at a level of around £500 million each year.

Seeding drug discovery is a new Wellcome Trust funding stream for early stage drug discovery, where it can be hard to attract funding from industry and government agencies. "We're very excited about this £91 million initiative," says the Wellcome Trust's Director of Technology Transfer Ted Bianco. The initiative covers unmet medical needs such as malaria and tuberculosis as well as orphan drugs and ideas that need a strong proof of concept.

Around 3,300 researchers are supported by Medical Research Council (MRC)–funded programmes. In fiscal year 2005–2006 more than £50 million was spent training researchers in universities and hospitals, through 350 fellowships, 30 New Investigator Awards, and around 420 postdoctoral studentships. Although funded by government, the MRC is independent in its choice of which research to support.

Clusters of high tech industry can be found in the UK's university science parks. continued »

UPCOMING FEATURES

Careers in Chemistry — August 10

Postdoc Scientists 2: Survey — August 31

Faculty Positions 2 — September 14

"Our future prosperity rests more than ever before on the hard work and genius of our scientists and how we harness their research to deliver improvements in all our lives."
— Prime Minister Tony Blair

Finland's Nokia has just unveiled plans to spend £40 million on a nanotechnology research center in Cambridge, a "nerve center" from which the next generation of mobile phone technology, including wearable electronics, will emerge. **Mark Welland,** who heads Cambridge University's Nanoscience Centre, says Nokia is hiring Ph.D. and postdoctoral staff immediately, and is doubling the number of university-based Nokia projects. "Nokia does get involved in real collaborations," he says. "They can see opportunities amongst the other companies already active here, as well as the potential for spin-out companies to work with in future."

One UK infrastructure investment eagerly anticipated by the research community is the Diamond synchrotron light source, a new £260 million facility which opened its doors in February. Funded by the government and the Wellcome Trust, Diamond is the largest scientific facility built in the UK for 30 years. "Our future prosperity rests more than ever before on the hard work and genius of our scientists and how we harness their research to deliver improvements in all our lives," Prime Minister Tony Blair said when touring the facility last November. "This is exactly what Diamond Light Source will help us achieve in many fields, from developing new drugs to tackling climate change."

Getting Physical

Although nanoscience that includes a bio element is extremely popular, academic career prospects in hard physics or nanoelectronics are more limited. Physics departments, in particular, are heavily dependent on public funds to support their research, according to **Tajinder Panesor,** manager of science policy at the Institute of Physics (IOP).

An IOP survey showed that less than 5 percent of physics departments' research income came from EU sources. Applying for European funding has involved a lot of red tape, a situation that should improve under the new European Seventh Framework Programme (FP7)—the world's biggest single, publicly funded research effort and the main funding mechanism behind collaborative research and technological development in the EU.

"Hopefully more physicists will apply for funds under the FP7 themes," says Panesor. **Sean McWhinnie,** science policy manager at the Royal Society of Chemistry (RSC), says FP7 is more specifically oriented toward chemistry, including nanotechnology, materials science, and energy, than the previous program, FP6. "I'm not going to deny that being bio oriented helps these days—but in FP7 there are more opportunities if you're prepared to look."

McWhinnie adds that the level of support for university Ph.D. positions, as envisioned in Ireland, is not mirrored in the UK. "Since [Prime Minister] Margaret Thatcher, technical staff have been ripped away." McWhinnie believes, based on statistics from HESA (Higher Education Statistics Agency), that it is still relatively easy to get funding for a Ph.D. in chemistry, which has the largest Ph.D. output of any UK subject.

Funding Woes

The Department of Trade and Industry, which handles the public science budget, recently redirected £68 million from research council (state funding agency) budgets to nuclear power company British Energy and car firm MG Rover, to the dismay of the research community.

The Engineering and Physical Sciences Research Council (EPSRC), for example, had to cut £29 million from its funding programs. "Decisions have inevitably impacted on the seed corn of the future: innovative research and young people at the start of their research careers," said EPSRC's Interim Chief Executive **Randal Richards.** The EPSRC decided to protect research studentships at the expense of research grants, and has thus been forced to reduce award grants by £14 million for 2007–2008.

On a more bracing note, UK Chancellor Gordon Brown reaffirmed long-term support for UK science with a budget announcement that public science spending would rise to £6.3 billion by 2010 from today's figure of £5 billion.

Fellowships

Postdoctoral research assistant positions generally involve the uncertainty of short-term contracts, a career phase that can last for up to 10 years before scientists obtain a permanent academic position.

In the UK junior faculty positions (lectureships) are highly sought after and only achieved by a minority. "There are far more postdocs than lecturing posts available," says McWhinnie of the RSC. continued »

BioIndustry Association
www.bioindustry.org

Diamond Light Source
www.diamond.ac.uk

EPSRC
www.epsrc.ac.uk

Institute of Physics
www.iop.org

Nanoscience Centre Cambridge
www.nanoscience.cam.ac.uk

Oxford University
www.careers.ox.ac.uk

Research Councils UK
www.rcuk.ac.uk

Royal Society of Chemistry
www.rsc.org

Science Foundation Ireland
www.sfi.ie

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Success without boundaries

"We were net exporters of researchers a few years ago, but that's changed now and we're bringing people back into the country."
—Desmond Fitzgerald

"A postdoc may be seen as a way of widening your experience or an opportunity to travel. But it isn't a ticket to a lectureship." The government's Academic Fellowships programme gives researchers a leg up with up to one thousand funded university positions worth £25,000 annually for five years. The fellowships offer a more attractive and stable path into academia, with the added bonus of a permanent academic position after the five years is up. Universities or other sources, such as a Research Council grant or a fellowship awarded by another sponsor, supplement the funding.

Fellowships are also available through the new Science and Technology Facilities Council, launched in April and having a budget of ~£500 million. With a physical science remit, it was formed from the merger of the Council for the Central Laboratory of the Research Councils (CCLRC) and the Particle Physics and Astronomy Research Council (PPARC). "We have a huge opportunity to develop a really coherent strategy for 'big science,' to increase our influence in international organizations and make a step change in the exploitation of the resulting technology," said professor **Keith Mason,** the council's new chief executive officer.

Ireland's Influx

Tourists have always looked to Ireland for its warm welcome and beautiful scenery. Today Ireland is also greeting an influx of scientists and major multinational companies, keen to take part in one of Europe's biggest economic success stories.

Ireland initiated the largest investment in scientific research and engineering in its history in 2000 by founding Science Foundation Ireland (SFI), a major step toward joining the knowledge-based economy set out in the Lisbon Agenda.

As part of its National R&D action plan, Ireland aims to invest 2.5 percent of GNP on R&D by 2010, with two-thirds contributed by industry. Recruiting the best research talent from around the world remains a challenge, although Ireland's profile as a research location has been noted by major investors such as Wyeth, Bell Laboratories, and IBM, all of which have established research facilities there.

Gary Crawley heads SFI's frontiers engineering and science directorate, and says that the strategy to lure information and communications technology (ICT) and biopharmaceutical companies to Ireland has paid off. "Now we're encouraging those same international companies to engage in research in Ireland. A number of those, such as IBM and Intel, are contributing to large center grants run by SFI." Collaborations typically include an industry cash contribution of 20 percent, and providing scientists to work with university-based participants.

Professor **Desmond Fitzgerald,** vice president for research at University College Dublin (UCD), cites Intel as a prime example. "Intel has just announced a major R&D program, part of which is with the universities," Fitzgerald says.

Ireland's inward investment body, the Industrial Development Agency (IDA), supported 54 R&D investment projects in 2006 involving a total of almost €470 million. According to the IDA, employment in IDA-supported companies increased by 3,795 in 2006, bringing the total employment to 135,487.

IBM was one of the first multinational companies to invest in Ireland 50 years ago. The company's latest investment is an expansion of its IBM Tivoli software development labs in Cork and Galway, where it will create up to 130 new positions over three years. Meanwhile, indigenous Irish ICT company Silicon and Software Systems is to create 20 new R&D roles in Dublin and Cork.

GlaxoSmithKline employs over 1,600 people at four sites in Ireland, and has now announced a five-year investment of €250 million in a production site expansion at Currabinny, County Cork, for its breast cancer treatment Tykerb. The move will create up to 150 high level jobs.

California biopharmaceutical company Gilead Sciences has recently announced that it will invest €60 million in a new pharmaceutical plant at Grange Castle Business Park near Dublin. The company has outgrown its existing Irish facility at Sandyford. Gilead will join Wyeth, the fastest growing biotechnology company in Europe, which has located one of the world's largest integrated biotech production facilities at Grange Castle. The investment had continued »

Tips for Success

Denise Best, careers adviser for postgraduate research students at Oxford University Careers Service, says that although there's a feeling that doing a postdoc in another country helps your career, nothing beats getting a few decent publications under your belt and working in a research group with a good reputation. "Actually learning something about project management might help you become more efficient in your research and give you more time to spend on getting published." She also recommends workshops on writing to improve one's chances with grants and publications. Oxford's Skills Portal website (www.skillsportal.ox.ac.uk) keeps campus researchers abreast of the development opportunities on offer, and most universities are putting money into improving training for Ph.D.s. "Determination and focus are key elements to success in research," she says.

a price tag of almost $2 billion and made Wyeth Ireland's largest pharmaceutical employer.

Brian Fitzpatrick, associate director for analytical sciences at the site, joined Wyeth after working at a small clinical trials company. Wyeth took him to the US and UK for training before Fitzpatrick implemented what he had learned at Grange Castle. Fitzpatrick's enthusiasm for working in an industry team is palpable: "You're engaged with the academic community and you have hands-on opportunity to shape processes and molecules."

Fitzpatrick has seen the biotech scene change dramatically in less than a decade. "Grange Castle was the first mammalian cell culture facility in Ireland, so previously there weren't really a huge volume of opportunities." Back then, all of his postgraduate lab colleagues traveled overseas to seek out opportunities. "I think the horizon in Ireland is very different now. We've gone from being a small-molecule center to having a biotech focus—six years ago there was none of that." Now, researchers can take their pick: "For people with good degrees and experience there are limitless opportunities," says Fitzpatrick.

However, the country's Strategy for Science, Technology and Innovation 2006-2013 document admits that Ireland's foundations are weak compared with other leading nations like Sweden or the US. Ireland is starting from a much smaller research base than many European countries. "It's difficult to invest too quickly in science, because you waste money that way," says Crawley. "You really need to build infrastructure, and people who can carry out the research, and that all takes time."

To further its scientific ambitions, Ireland wants to boost the number of people with advanced science and engineering qualifications, and develop its international profile as a world class R&D location. Research funding in Ireland has more than doubled in five years to exceed €680 million in 2005. SFI has specifically targeted high-level researchers in a number of its funding programs.

Skills Boomerang

In previous years, emigration was often the first step on the Irish researcher's career ladder. "Twenty-five years ago many Irish people with good skills had to leave because of the lack of jobs," Crawley explains. "In some ways that's turning out to be an advantage, because a number of people would like to come back, including senior professors."

SFI has Research Professor Recruitment Awards that include a startup package of up to €1 million. "In the US, if you were trying to recruit even a junior person you might have to pay as much as half a million or a million dollars as a startup fund. We are going to step in and help Irish universities attract really top class people from outside Ireland with these grants," Crawley continues. Around 50 percent of SFI's funding goes to non-Irish researchers or researchers returning to Ireland from abroad. SFI has also just launched the new Stokes Professorships and Lectureships to attract faculty from outside Ireland. These are available for biomedical science, ICT, and mathematics. Fitzgerald has witnessed the tide begin to turn: "We were net exporters of researchers a few years ago, but that's changed now and we're bringing people back into the country."

Jointly funded research studentships for industry-relevant research have grown rapidly, helped by a strong Irish tradition of doctoral training funded by or for industry. Examples are the Irish Research Council's Embark Cooperative Awards and SFI Centres for Science Engineering and Technology (CSET). The latter help to fund partnerships across academe and industry to aid new and existing Irish-based technology companies with grants of €1 million to €5 million each year for five years.

"You really need to build infrastructure, and people who can carry out the research, and that all takes time."
—Gary Crawley

Part of the Irish strategy to double its output of Ph.D.s includes the funding of supporting roles in research teams. There are plans to add 350 principal investigator positions and 1,050 postdoctoral researchers by 2013 as well as increasing the pool of research assistants and technicians. This move has considerable support, and will arguably make Ireland an attractive location to undertake a Ph.D.

Historical deficits in infrastructure are being remedied by investment in state-of-the-art facilities. Major initiatives include the National Institute for Bioprocessing Research and Training (NIBRT). Construction is under way at University College Dublin's Belfield campus and should be completed by 2008, with a strong focus on applied research. The university will also be home to the National Digital Research Centre (NDRC). Appropriate space and technology are a challenge everywhere, Fitzgerald says. "But we have a lot of catching up to do to be on a par with top universities in Europe and the US."

Keeping Your Options Open

What academic communities in both the UK and Ireland have in common is a wide range of opportunities for postgraduate and postdoctoral researchers, but a limited number of academic positions to move into. "Those young people have to realize and look at career paths outside of academia—only a small fraction of them will finish up doing research in universities," says Crawley.

Good quality industry researchers are in demand, especially those with a flexible outlook and good interpersonal skills. **Tony Bradshaw** from the BioIndustries Association says that pharmaceutical development is just one sector where there are fantastic opportunities and a shortage of applicants. But he thinks the smartest UK applicants should consider openings in academia as well as biotech and pharma. "You'd be really competitive if you could work in all three at some point in your career. There are roles to play at that interface."

Can Lynch see himself in academia for the long haul? "You have to—there's no point staying in academia unless you think you can get funding and run your own lab." Lynch is undeterred by a future at the thin end of the academic wedge. "Sequencing the human genome—that was only the first version and it was like planting a flag on the moon. There's just so much more to do."

Helen Carmichael is a freelance writer living in Dorset, UK.

International Careers Report: Germany

Whether recruiting, creating awareness, repositioning your organisation, or promoting your products and services, don't miss the opportunity to be seen in this issue.

Issue Date:
21 September 2007

Booking Deadline:
4 September 2007

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Email: j.quinn@chesterzoo.org

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To take this first step, all you need to do is email us at roslin.hr@bbsrc.ac.uk with your CV and a covering note. We'll do the rest.

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Tenure-Track Positions
Liver Diseases Branch

New Research Initiative – Fatty Liver Disease & Obesity - Tenure Track Position:
The Liver Diseases Branch of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH) invites applications for one tenure track position from scientists interested in basic and/or clinical research involving non-alcoholic fatty liver disease and metabolic syndrome. Specific areas of research interest include pathogenesis and mechanism of metabolic derangement in non-alcoholic fatty liver disease and its pathophysiological link to insulin resistance and obesity. Priority will be given to applicants at the Assistant Professor level in traditional universities or those finishing their post-doctoral/fellowship positions.

New Research Initiative – Liver Stem Cells - Tenure Track Position:
The Liver Diseases Branch of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH) invites applications for one tenure track position from scientists interested in basic and/or clinical research involving mammalian adult stem cells. Specific areas of research interest include functional differentiation and mechanism of development of adult tissue-derived stem cells, especially those of the liver, and potential clinical application of stem cell therapy in liver diseases. Priority will be given to applicants at the Assistant Professor level in traditional universities or those finishing their post-doctoral/fellowship positions.

The applicant must have a proven record of accomplishments and will be expected to propose and pursue an independent research program in one of these fields. The position offers unparalleled opportunities for interdisciplinary collaboration within NIDDK and throughout NIH. The Liver Diseases Branch of NIDDK is located on the main intramural campus of the NIH in Bethesda, Maryland, a suburb of Washington, D.C.

Interested applicants should send a Curriculum Vitae and list of publications, copies of three major publications, a summary of research accomplishments, a plan for future research, and three letters of recommendation to **Ms Michelle Brown, Search Committee, Liver Diseases Branch, NIDDK, Building 10-9B16, NIH, Bethesda, MD. 20892-1800**. Application deadline: **September 15, 2007**.

Division of Extramural Research
Program Director

The National Institute of Neurological Disorders and Stroke, NIH, is seeking a scientist or physician with experience in Prion Diseases, Infectious Diseases, Neurovirology, or Neuroimmunology to serve as Program Director for the planning, evaluation, and administration of research on Infectious Diseases of the Nervous System. Successful candidates will join a highly interactive group of scientists and clinicians directing research programs in all areas of modern neuroscience and neurological disorders. He/she will plan and implement a cutting edge research program through active communication with the professional and lay communities as well as program staff from other institutes and agencies.

Applicants should have a Ph.D. and/or M.D. degree in a relevant field of biomedical science. This is a GS 13/14/15 civil service position (salary range $ 79,397.00 - $ 143,471). Physicians may be eligible for a Physician's Comparability Allowance.

Applications may be submitted beginning May 14th through July 13, 2007. Beginning **May 14th**, official application instructions can be found at the USAJobs Web Site (**http://www.usajobs.gov/**), by searching on Vacancy Announcement **NINDS-07-187491 for the Health Scientist Administrator and NINDS-07-188681 for the Medical Officer**. For further information about the application process, please contact **Sharon Scott (scottsha@mail.nih.gov)** at NIH Human Resources. For more details concerning the nature of this position, please contact **Dr. Emmeline Edwards (edwardse@mail.nih.gov)**.

NIDDK

HEALTH SCIENCE POLICY ANALYST
(Two Positions Available)

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is seeking applications from individuals who are currently in post-doctoral positions in biomedical research laboratories, but who wish to make a career change from a laboratory setting. Particularly encouraged to apply are individuals with post-doctoral experience in molecular biology, coupled with demonstrated writing and other communication skills. Incumbent will develop a wide range of documents that analyze and present the scientific accomplishments and plans of the NIDDK to public policy makers, voluntary health organizations, and other lay audiences. Incumbent must thus be able to convey in understandable, scientifically accurate, and meaningful terms the contributions of biomedical research to human health. Total salary is competitive and will be commensurate with the experience of the selectee.

Position requirements and detailed application procedures are provided on Vacancy Announcement Numbers: **NIDDK-07-197281-DE and NIDDK-07-197281-MP**, which can be obtained by accessing **WWW.USAJOBS.GOV**. All applications must be received by **07/20/07**. For additional information contact **Karen Page, Human Resources Specialist at (301) 496-4232**.

WWW.NIH.GOV

NIDDK

Tenure-Track Position in Molecular Genetics
National Institutes of Diabetes and Digestive and Kidney Diseases

We seek an outstanding scientist to direct a vigorous, innovative research program in Molecular Genetics in the Genetics and Endocrinology Section/Metabolic Diseases Branch. Applicants must have a demonstrated track record of significant publications that address identification and mechanisms of action of tumor genes. The successful candidate is expected to develop an independent, world-class research program complementary to current investigations within the Branch. The position comes with generous start up funds and on-going support.

The Metabolic Diseases Branch of NIDDK is located on the main NIH campus in Bethesda, Maryland, a suburb of Washington DC. The Branch represents interests similar in range to those of an academic department with groups studying G-proteins and hormone-secreting tumors including those mediated by the MEN1 or HRPT2 genes in man. There are strong interactions among the three independent research groups, and the position offers unparalleled opportunities for interdisciplinary collaboration within NIDDK and throughout NIH. Applicants should submit a curriculum vitae, bibliography, copies of three major publications, a summary of research accomplishments, a brief statement of future research goals, and arrange for three letters of reference to be sent to:

Dr. Dan Camerini-Otero, Chair, Search Committee, c/o Linda Robinson, NIDDK, 9000 Rockville Pike, Building 5/Room 201, National Institutes of Health, Bethesda, MD 20892.

Application Deadline: **June 15, 2007**.

NIDDK

Tenure-Track Position in Human Molecular Genetics
National Institute of Diabetes and Digestive and Kidney Diseases

We seek an outstanding scientist to direct a vigorous, innovative research program in the molecular genetics of human type 2 diabetes and/or obesity, in particular as these diseases relate to the Pima Indian population of Arizona. Applicants must be highly motivated and have a demonstrated track record through publications that address significant issues of discovery of genetic susceptibility factors to these conditions in human populations. The successful candidate is expected to develop an independent, world-class research program complementary to current investigations within the Phoenix Epidemiology and Clinical Research Branch (PECRB). The position comes with generous start up funds and on-going support.

The PECRB, NIDDK is located in downtown Phoenix, Arizona. The Branch represents interests similar in range to those of an academic department. There are strong interactions among the independent research groups, and the position offers unparalleled opportunities for interdisciplinary collaboration within NIDDK and throughout NIH. Applicants should submit a curriculum vitae, bibliography, copies of three major publications, a summary of research accomplishments, a brief statement of future research goals, and arrange for three letters of reference to be sent to:

Dr. Dan Camerini-Otero, Chair, Search Committee, c/o Linda Robinson, NIDDK, 9000 Rockville Pike, Building 5/Room 201, National Institutes of Health, Bethesda, MD 20892.

Application Deadline: **June 15, 2007**.

BIOMEDICAL INFORMATICS FACULTY POSITION

The H. Lee Moffitt Cancer Center and Research Institute in conjunction with the College of Medicine at the University of South Florida, Department of Interdisciplinary Oncology, are seeking applicants at the Assistant/Associate Professor level. The successful candidate will pursue independent and collaborative research in the fields of bioinformatics and oncology, especially focusing on the development of computational and mathematical methods to reveal specific data signatures targeted at diagnostics, prognostics and personalized therapies. The successful applicant will propose and develop a research theme consistent with ongoing projects at the Moffitt Cancer Center. Applicants with a strong background in data mining expertise using high-density gene expression, proteomics and clinical data will be of particular interest. State-of-the-art core facilities including multiple supercomputing facilities, vigorous research programs and access to large heterogeneous data resources are among the many strengths of the research environment at Moffitt.

Position includes generous start-up package as well as ongoing commitment of space and access to computing resources. As one of the fastest-growing NCI Comprehensive Cancer Centers with the third largest cancer hospital in the nation, major investments are being made at Moffitt in the areas of microarray gene expression profiling and proteomics, with an emphasis on personalized cancer care. Candidates must have a Ph.D. or M.D. degree with advanced postdoctoral training, and a record of accomplishment in methodological development for knowledge discovery from databases, systems biology or biomedical informatics as evidenced by publications.

Successful individuals for Assistant Professor must have at least two years research experience in bioinformatics or related fields. The Associate Professor must have a proven track record of independent research and demonstrated sustained extramural funding. In addition, the Associate Professor rank requires at least five years of experience with continuing and productive service as an Assistant Professor. The position may be tenure earning and salary is negotiable.

Please refer to position no. 15119. Send curriculum vitae, brief research plan, current and past grant support and names of three references to David Fenstermacher, Ph.D., Executive Director, Research Informatics, c/o Kathy Jordan, MBA, Supervisor Recruitment & Appointments, Department of Interdisciplinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612. For inquiries contact Dr. Fenstermacher at 813-745-6711 or david.fenstermacher@moffitt.org. Electronic CVs preferred to kathleen.jordan@moffitt.org. Position is open until filled. Application review begins July 1, 2007.

H.LEE MOFFITT Cancer Center & Research Institute

The End Of Cancer Begins Here.

A National Cancer Institute Comprehensive Cancer Center At the University of South Florida

USF HEALTH

USF Health is committed to increasing its diversity and will give individual consideration to qualified applicants for this position with experience in ethnically diverse settings, who possess varied language skills, or who have a record of research that supports/benefits diverse communities or teaching a diverse student population. The University of South Florida is an EO/EA/AA Employer. For disability accommodations, contact Kathy Jordan at (813) 745-1451 a minimum of five working days in advance. According to FL law, applications and meetings regarding them are open to the public.

www.moffitt.org

TECHNISCHE UNIVERSITÄT DRESDEN

Position Project Manager

Mission: Fondation Leducq, a Paris-based nonprofit institution, has awarded a grant through its 2006-2007 "Transatlantic Networks of Excellence Program" to the "**Leducq European-North American Atrial Fibrillation Research Alliance**". The research network http://www.transatlantic-af-alliance.org/ will receive $6 million over five years to support a collaborative research program on the role of calcium in atrial fibrillation involving leading European and North American investigators.

Position: A position for a Project manager will be available at the coordination site in Dresden from October 01, 2007 for an initial period of one year, renewable for up to four years up to September 30, 2012.

Qualifications: Coordination of all scientific and administrative activities; development, planning, and implementation of communication strategies between the centers and between the coordination site and Fondation Leducq; development and implementation of administrative tools for planning and control of budgets; supervision of budget allocation to the network centers working in tight collaboration with the coordinators and the budgetary administration of the Medical Faculty; organisation of the annual network meetings and scientific conferences; management of personnel exchange; preparation of timely annual scientific and budget reports to be presented to Fondation Leducq; administrative support of the coordinators, etc.

Requirements: Life science degree (or equivalent); fluent verbal and written English; excellent analytical, problem-solving, initiative, decision-making, and communication skills; strong organizational skills with ability to coordinate activities across various centers; advanced computer skills and ability to learn new systems; previous experience in administration of research projects or closely related fields.

Interested individuals should send an application letter and curriculum vitae by August 1st to: **Dr. Dobromir Dobrev, Medizinische Fakultät Carl Gustav Carus, Institut für Pharmakologie und Toxikologie, TU Dresden, Fetscherstr 74, 01307 Dresden, Germany** (mail) or per e-mail to: **dobrev@rcs.urz.tu-dresden.de**.

UT SOUTHWESTERN MEDICAL CENTER

FACULTY POSITIONS

DIVISION OF BIOMEDICAL INFORMATICS DEPARTMENT OF CLINICAL SCIENCES UT SOUTHWESTERN MEDICAL CENTER

The Division of Biomedical Informatics in the Department of Clinical Sciences at UT Southwestern Medical Center in Dallas, Texas is seeking applications for Assistant Professor/Associate Professor/Professor level faculty, the academic rank of which will be determined by the qualifications of the candidate.

Doctoral level candidates for this position should demonstrate a strong professional desire to advance and strengthen the fields of bioinformatics and computational biology as applied to clinical and translational research as evidenced by professional experience and accomplishments. This would include innovative contributions to peer-reviewed publications, obtainment of or active participation in extramural research funding, involvement on academic/institutional committees, and successful teaching in interactive and thought-provoking coursework at the graduate level. Areas of research interest should include one or more of the following: clinical and translational data modeling, biomedical ontology development, algorithm development and testing, data analysis and data mining. The candidate must have an excellent interpersonal and communication style essential for developing and building collaborative research projects throughout the medical environment.

If interested, please send a letter of interest and your C.V. to:
Richard H. Scheuermann, Ph.D., Professor and Chief, Division of Biomedical Informatics, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75390-9072;
e-mail: Richard.Scheuermann@utsouthwestern.edu

UT Southwestern Medical Center is an Equal Opportunity Employer

World Meteorological Organization

DIRECTOR
WORLD CLIMATE RESEARCH PROGRAMME

The World Meteorological Organization (WMO) invites applications for the post of Director of the World Climate Research Programme (WCRP) Department. The incumbent is responsible on behalf of the sponsoring bodies (WMO, ICSU and IOC of UNESCO) for the international coordination, planning and organization of scientific research projects and related activities contributing to the goals of the WCRP programme. The incumbent represents the interests of the programme with relevant governmental and non-governmental international organizations and a wide range of national administrations and research agencies. He/she will establish effective working relationships with a wide international community of scientists in the fields of meteorology and atmospheric sciences, oceanography, polar sciences, hydrology and land surface processes, as well as space research. In accordance with the terms of the WMO/ICSU/IOC Agreement on the WCRP, the incumbent will be responsible for the scientific and technical tasks to the Chairperson of the Joint Scientific Committee for the WCRP and for financial and administrative matters, to the Secretary-General of WMO.

Applicants will have Ph.D or equivalent qualification in the fields meteorology, atmospheric sciences, oceanography, polar sciences, hydrology or space science. Over fifteen years experience including international research recognition in climate-related studies and global environmental change. Experience in planning and organizing large scientific projects and/or management of a scientific institute preferably with international components. Demonstrated ability in resource mobilization. Excellent knowledge of English and/or French and a good working knowledge of the other.

Deadline for applications: 10 August 2007.

For Personal History Forms, as well as additional information concerning the position, salary and benefits: **www.wmo.int/web/hrm.**

Interested candidates should send a cover letter and Personal History form addressed to the Secretary-General, World Meteorological Organization, P.O. Box 2300, 1211 Geneva 2, Switzerland.

POSTDOCTORAL FELLOWSHIP PROGRAM

NATIONAL SPACE BIOMEDICAL RESEARCH INSTITUTE

The National Space Biomedical Research Institute (NSBRI) is soliciting applications for its Postdoctoral Fellowship Program. Two-year Fellowships are available in any U.S. laboratory carrying out space-related biomedical or biotechnological research that supports NSBRI's mission and objectives.

Applicants must submit proposals with the support of a mentor and institution, and all proposals will be evaluated by a peer-review panel. *The Program is open to U.S. citizens, permanent residents, or persons with pre-existing visas obtained through their sponsoring institutions that permit postdoctoral training for the project's duration.*

Detailed program and application submission information is available on the NSBRI website at **www.nsbri.org/Announcements/rfa07-02.html**. Notices of intent and applications must be submitted through an electronic proposal submission system. *Notices of intent are due July 10, 2007, and the application deadline is August 1, 2007.*

Questions to: Sonia Rahmati Clayton, Ph.D., Program Coordinator, NSBRI Postdoctoral Fellowship Program, telephone: 713-798-8229, email: postdoc@www.nsbri.org.

RIGEL

Rigel Pharmaceuticals, Inc. *is a biotech company that discovers and develops novel, small-molecule drugs for the treatment of inflammatory diseases, cancer and viral diseases. We have several opportunities in South San Francisco for dedicated, hands-on professionals to join our multi-disciplinary R&D teams.*

SCIENTIST, VIROLOGY
Requires a Ph.D. in the biological sciences, 3+ years of relevant postdoctoral/industry experience, and a track record of scientific accomplishments in retrovirus biology. ***(Job Code: YJ-06-07-SCI)***

SCIENTIST, HTS
Requires a Ph.D. in biological sciences, 3+ years of experience in the area of molecular mechanisms of obesity and diabetes research, and experience in molecular & cellular biology and biochemistry. ***(Job Code: XXU-03-2-SCI)***

SCIENTIST, DISCOVERY
Requires a Ph.D. in biological sciences, 2+ years of post-graduate experience, and broad scientific backgrounds spanning multiple fields. ***(Job Code: TK-06-05-SCI)***

ASSOCIATE SCIENTIST/SENIOR SCIENTIST I, TOXICOLOGY
Requires a BS/MS/DVM/PhD in Biology, Pharmacology, Toxicology or Laboratory Animal Sciences, 2+ years of research experience in a pharmaceutical-oriented environment, and the ability to independently design, set up, initiate, undertake, terminate, and report in vivo Toxicology/DMPK or Pharmacology studies. ***(Job Code: GC-06-07-SCI)***

At Rigel, not only will you enjoy our exceptional compensation & benefits package, our generous stock options, and the camaraderie between all of our valued employees, but you'll also have complete access to the most advanced scientific equipment & latest technology as well as the opportunity to grow as a person AND as a member of our growing interdisciplinary teams. Reference the corresponding Job Code and email resume to: **careers@rigel.com**. *EOE*

VIEW COMPLETE JOB DESCRIPTIONS AT: **www.rigel.com**

IRRI
INTERNATIONAL RICE RESEARCH INSTITUTE

DEPUTY DIRECTOR GENERAL FOR RESEARCH

(Ref.: DDGR 03/07)

The International Rice Research Institute (**www.irri.org**) is seeking a Deputy Director General for Research as a member of its senior management team at its headquarters in Los Baños, Philippines. This position has full responsibility for leading, planning, executing, managing, and monitoring IRRI's global research for development activities through a research management matrix system of programs and disciplines. Interested candidates should have a PhD in agricultural, natural, or social sciences; an outstanding record of research; and strong leadership ability in research planning and management. A complete position description and information about IRRI can be found at **www.irri.org/irsjobs.htm** or contact IRRI's HR coordinator, Ms. Selene Ocampo at **s.ocampo@cgiar.org.**

Do you have a University degree in Biological/Life Sciences, Biochemistry or Engineering and several years of experience in IVD or pharmaceutical reagent manufacturing/QC?

Our client is one of the world's most successful and rapidly growing life science companies with HQ in Switzerland and over 20'000 staff members worldwide. The company's new

Center of Competence

for **PCR**-systems used in diagnostics and research, located in the greater Zurich/Lucerne area, has an exciting and challenging opening for a

Head Operations Molecular Systems

Reporting to Top Management you are responsible to establish and head a multi-disciplinary operation engaged in bulk formulation and filling, kit packaging, QC testing, validation, logistics, and product distribution. In this pivotal role you manage a group of approx. 30 academic and technical employees. The ideal candidate has already led projects to success and possesses extensive

Leadership

experience. Strong communication skills and an excellent command of English and German are a must.

If you are interested in this outstanding career opportunity within a very strong global company, we are looking forward to receiving your letter of application with CV and letters of reference.

Your application will be treated with strict confidentiality.

PP PHARMA PLANING
Dr. Bergauer + Partner GmbH
International Health Care Recruitment

Ms Regina Sager-Desserich
MA Law
direct dial: +41-61-261 94 17
Gerbergässlein 41
CH-4051 Basel
r.sager@pp-pharma-plan.com
www.pp-pharma-plan.com

VERTEX

Vertex Pharmaceuticals Incorporated is a global biotechnology company committed to the discovery and development of breakthrough small molecule drugs for serious diseases. The Company's strategy is to commercialize its products both independently and in collaboration with major pharmaceutical companies. Vertex's product pipeline is focused on viral diseases, inflammation, autoimmune diseases, cancer, pain and bacterial infection.

Research Scientist

This position is responsible for providing scientific lab leadership to support the initiation and execution of drug discovery and technology projects focused on membrane proteins. The Research Scientist will be expected to work with the biology team to advance new molecular approaches for ion channel analysis, using biochemical and biophysical methods. Responsibilities will include coordinating, designing and executing research strategies involving scientists from multiple departments to probe drug-target interactions and target biology.

We are in search of a candidate possessing a degree in Neuroscience, Biochemistry, or a related field, and 3+ years of post-doctoral experience. The successful candidate will have an excellent scientific track record, communication skill, and substantial experience with membrane proteins.

For consideration, please submit your resume online at: **www.vrtx.com**.

Vertex Pharmaceuticals Inc., is an EQUAL OPPORTUNITY EMPLOYER.

UNIVERSITY OF OULU, FINLAND
Faculty of Science

Applications are invited to the position of
Full Professor in Cellular Biochemistry

The position is open in the Department of Biochemistry. The appointee should have a strong research program in the field of cellular biochemistry (for example cell signalling, cell trafficking, assembly of macromolecular complexes, organization of metabolic pathways and their regulation). The teaching duties will include both basic and advanced courses at the graduate level and also at postgraduate level in the candidate's field of expertise. The lectures at the Department are given in Finnish or English.

The Department of Biochemistry houses well-equipped laboratories for research in molecular biology, protein chemistry, biocomputing and structural biology. Laboratories for cell biology are being reconstructed. The Department provides a stimulating, multidisciplinary international environment with world-class level research and recognized excellence in teaching. The starting salary will be based on the demands level chart for teaching and research personnel in the salary system of the Finnish universities (levels 8-10). In addition, the appointee will be paid a salary component based on personal work performance.

The detailed description of the post is available at **http://www.hallinto.oulu.fi/yhallint/kuulutus/virat.html.** *Informal information can be obtained* email: **kalervo.hiltunen@oulu.fi** or tel: +358-8-553-1150. *Further information and application instructions please contact* by email: **pertti.tikkanen@oulu.fi** or tel: +358-8-553-1051.

Applications including the documents required according to the application instructions should be received *no later than July 9th, 2007 at the University of Oulu, Registrar Office, P.O.Box 8000, FIN - 90014 Oulu University, Finland.*

Postdoctoral Positions in the Genetics of Susceptibility to Prion Diseases and other Neurodegenerative Disorders

McLaughlin Research Institute is a small non-profit research organization near the east slopes of the Rocky Mountains and provides an outstanding environment to train for a career in mammalian genetics. Applicants for these positions should provide evidence, including publication in internationally recognized journals, for their potential for an independent research career.

The positions are supported by a NINDS Program Project led by George Carlson, Director of the Institute. The goal is to identify genes and mechanisms that modify susceptibility to prion infection or that are involved in prion replication. Emphasis will be placed on exploiting a newly developed CNS stem cell culture system that can be infected with prions. Interactions with co-investigators at larger research centers in Seattle (**L.E. Hood**) and San Francisco (**S.B. Prusiner** and **S.J. DeArmond**) enhance the training environment. Successful applicants will have a solid background in molecular genetics or stem cell biology.

McLaughlin Research Institute for Biomedical Sciences

To apply, send your curriculum vitae, a statement of research experience and interests, and the names of three individuals we may contact for references to:

Training Office
McLaughlin Research Institute
1520 23rd Street South
Great Falls, MT 59405
tg@po.mri.montana.edu

ZFIN
The Zebrafish Information Network

ZFIN, the zebrafish model organism database, seeks a scientific curator to join our dynamic, interactive team of biologists and computer scientists at the University of Oregon in Eugene, OR.

Scientific curation is emerging as an attractive, alternative career track for creative scientists. As a ZFIN curator you will:

- read the latest zebrafish literature, and add information about zebrafish genes, expression patterns and mutant phenotypes to the ZFIN database.
- work directly with research laboratories to incorporate their data into ZFIN.
- help integrate the emerging whole-genome sequence with ZFIN data.
- participate in working groups to facilitate cross-species comparisons.
- collaborate with computer scientists designing web interfaces to access scientific data.

Required:

- Ph.D. or M.Sc. degree in the life sciences.
- Excellent written and verbal English communications skills.
- Experience with developmental genetics.
- Familiarity with biological databases.
- Detail-oriented work style.

Send cover letter, curriculum vitae and references to:

Peg Morrow
Institute of Neuroscience
University of Oregon
Eugene, OR 97403-1254 USA.
Fax: 541-346-4548 Email: Morrow@uoregon.edu

Affirmative Action/Equal Opportunity/ADA Institution committed to cultural diversity.

Universität Potsdam

Max-Planck-Institut für Molekulare Pflanzenphysiologie

International Max Planck Research School in Primary Metabolism and Plant Growth Offers several doctoral fellowships starting fall 2007

The recently founded International Max Planck Research School (IMPRS) in Primary Metabolism and Plant Growth will focus on a systems oriented approach with an emphasis on both the application of a variety of molecular phenotyping technologies (Omics) and of bioinformatics, specifically on data integration and modelling. In this joint initiative of the Max Planck Institute of Molecular Plant Physiology and the University of Potsdam doctoral students will receive a high level and true interdisciplinary education covering genetics, cutting edge analytical techniques, and bioinformatics. Doctoral studies focus will to a large extend on the model organism Arabidopsis thaliana. The programme's language is English and no tuitions apply.

For information about the programme, the research groups and the online application procedure please visit our web page at:
http://www-en.mpimp-golm.mpg.de/IMPRS_GoFORSYS/index.html

Applications will be accepted until July 15, 2007

University Hospitals Case Medical Center

CASE
CASE WESTERN RESERVE UNIVERSITY SCHOOL OF MEDICINE

Chief, Endocrinology and Director, of the Diabetes Center

The Department of Medicine at Case Western Reserve University School of Medicine and University Hospitals Case Medical Center is seeking applicants for Chief, Division of Endocrinology and Director of the Diabetes and Obesity Center. The Division has 15 full-time faculty members providing patient care at University Hospitals Case Medical Center, the Cleveland Wade Park Veterans Administration Hospital, and outpatient satellite centers. The Division has current, robust basic, translational and clinical research programs. Its educational program includes University Hospitals Case Medical Center and the Wade Park Veterans Administration Hospital, with 5 fellows. The successful candidate will have an outstanding record of scholarly achievements, sustained extramural research funding, along with proven leadership, mentoring and administrative abilities. He/she should qualify for the rank of Professor with tenure at Case. A strong commitment to continuing to lead the Division in national prominence through building interdisciplinary programs as Diabetes and Obesity Center Director is expected.

Interested candidates should submit their curriculum vitae and a letter describing their research, teaching, service and administrative experience to: **Robert A. Salata, MD, Chair, Endocrinology Division and Director of the Diabetes and Obesity Center Chief Search Committee, Chief, Infectious Diseases, Department of Medicine, Case Western Reserve University, University Hospitals Case Medical Center, 11100 Euclid Ave., Cleveland, OH, 44106-5083**. Electronic format preferred to: **robert.salata@case.edu**.

Case Western Reserve University/University Hospitals Case Medical Center are Equal Opportunity/Affirmative Action Employers.

Max Planck Institute for Biogeochemistry
An Institute of the Max Planck Society

MPI-BGC Jena

The MPI for Biogeochemistry in Jena is committed to basic research on the role of biogeochemistry in the Earth System, with a special emphasis on terrestrial ecosystems. Founded in 1997, the institute has developed a research agenda comprising process studies, regional and global observations combined with theory and model development. The institute also collaborates closely with the MPI for Chemistry in Mainz, the MPI for Meteorology in Hamburg and the Potsdam Institute for Climate Impact Research in the German Earth System Research Partnership. The institute disposes over state-of-the-art technical facilities (chemical analysis, stable isotopes, gas analytics, field instrumentation, ^{14}C analyses, computing) and it has a leading role in major European research projects. (http://www.bgc-jena.mpg.de).

With the upcoming retirement of founding director, the institute will undergo a major reorganization and is looking for one or possibly more

Director(s) and Department Head(s) f/m

We are seeking outstanding individuals with broad research interests in global biogeochemical cycles. Possible fields to be covered include ecosystem processes, coupling of the major biogeochemical element cycles, hydro- and paleobiogeochemistry using experimental and theoretical approaches.

The positions are equivalent to a tenured full professorship at a German university. There are no teaching obligations; however, the Institute participates in various teaching activities at the University in Jena and is currently establishing a joint International Max Planck Research School on Biogeochemistry.

In this context, the Max Planck Institute for Biogeochemistry organizes a special 10th anniversary symposium on:

Earth System Dynamics: Biogeochemical Perspectives

to take place in Jena, September 24 - 26, 2007.

The Max Planck Society is an equal opportunity employer and specifically welcomes applications of female scientists. The Max Planck Society is committed to employing more handicapped individuals and especially encourages them to apply.

Scientists interested in this position and in participating in the symposium are requested to submit title and abstract of a possible presentation, a summary of their research plans, a CV and the list of publications **before July 31th, 2007** to

Professor Martin Heimann
Max Planck Institute for Biogeochemistry, PF 100164, D-07701 Jena, Germany
martin.heimann@bgc-jena.mpg.de

Participants in the symposium will be notified **before August 31th, 2007**.

Scientific Officers

The **Howard Hughes Medical Institute (HHMI)**, a national and international philanthropy devoted to biomedical research, is seeking a Scientific Officer (or Senior Scientific Officer) at our headquarters in Chevy Chase, MD. Scientific Officers have administrative responsibility for activities associated with HHMI's medical research operations at over 60 institutions across the country. These activities include participating in the management of HHMI's investigator competitions, investigator reviews, and science meetings and workshops.

The Scientific Officer (or Senior Scientific Officer) must possess a Ph.D. and/or M.D. plus at least 10 years research experience in the life sciences or the physical sciences, including experience as a principal investigator of grants, a record of publications in peer-reviewed journals, and some administrative experience. Travel to host institutions is required.

HHMI is an intellectually exciting organization with excellent salaries and benefits. Interested candidates should send curriculum vitae and bibliography, a brief statement of scientific experience and accomplishments **prior to August 15th**, either electronically to **glotfelt@hhmi.org** or by mail, to:

Jack E. Dixon, Ph.D.
Vice President and Chief Scientific Officer
Howard Hughes Medical Institute
4000 Jones Bridge Road
Chevy Chase, MD 20815-6789

The Howard Hughes Medical Institute is an Equal Opportunity Employer.

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

USC
UNIVERSITY OF SOUTHERN CALIFORNIA

ADVANCED BIOLOGY TRAINING COURSE IN ANTARCTICA
January 2008
"Integrative Biology and Adaptation of Antarctic Marine Organisms"

This National Science Foundation sponsored course will be held in Antarctica at the United States' McMurdo Station for one month, starting January 2008. This is an international course, open to all nationalities. Applications are invited from graduate students currently enrolled in a PhD program, postdoctoral fellows, and faculty-level research scientists who are interested in the study of extreme environments and the biology of Antarctic organisms. The course will accommodate up to 20 students. Full scholarships are available for each student accepted into the course to cover the cost of travel from home institution to Antarctica, and room and board while in Antarctica. The emphasis of the Antarctic Biology Course is on integrative biology, with laboratory- and field-based projects focused on adaptations in an extreme polar environment. A diverse teaching faculty will offer students the opportunity to study a wide range of Antarctic organisms (bacteria, algae, invertebrates, and fish), as well as studying several different levels of biological analysis (molecular biology, biomechanics, physiological ecology, species diversity, and evolution). Deadline for receipt of completed applications is **August 15, 2007**. For more information and on-line applications, please see -- **http://antarctica.usc.edu**.

OAK RIDGE National Laboratory

Associate Laboratory Director

NEUTRON SCIENCES

Oak Ridge National Laboratory (ORNL) is seeking an Associate Laboratory Director (ALD) for Neutron Sciences. The successful candidate will lead an organization of approximately 600 staff and $250M in annual funding for neutron science research in support of Department of Energy (DOE) missions. We are seeking an individual with demonstrated ability to manage large and complex research and development organizations, develop and implement strategic objectives, and provide national leadership on neutron science and technology issues. The position requires outstanding leadership, communications, strategic planning, management, and interpersonal skills. Broad experience in helping to develop and implement strategies at the national and DOE program levels is highly desirable.

The ALD is a member of the Laboratory senior management team, reporting to the ORNL Director. The position has line and program responsibility for the Neutron Sciences Directorate including developing and implementing a strategic vision, providing technical and management direction of the safe, reliable, and cost effective operation of facilities, and ensuring scientific and technical quality.

The ALD will provide the leadership to establish ORNL as the world's foremost center for neutron sciences, delivering unprecedented capabilities for understanding the structure and dynamics of materials. With the world's highest flux reactor-based neutron source - the High Flux Isotope Reactor (HFIR) and the world's most intense pulsed accelerator-based neutron source - the Spallation Neutron Source (SNS); Oak Ridge National Laboratory provides neutron scattering capabilities unavailable anywhere else in the world. The ALD will insure that SNS and HFIR will together comprise the world's leading neutron scattering capability with both pulsed and continuous beams as measured by instrument performance, scientific output, user satisfaction, predictability and availability of operations, and facility upgrades and expansions. The ALD will also cultivate a strong in-house research program, linked to other ORNL research areas and the broader research community, as a key element of a successful scientific user facility.

To learn more and apply, go to **http://jobs.ornl.gov** and select position number **2432**. Please submit your resume, references, and publication list in one file. We only accept Microsoft Word documents.

ORNL is a multi-program research facility managed by UT-Battelle, LLC for the Department of Energy. UT-Battelle, LLC is an equal opportunity employer and committed to building and sustaining a culturally diverse workplace.

www.ornl.gov

UMKC
UNIVERSITY OF MISSOURI-KANSAS CITY
SCHOOL OF BIOLOGICAL SCIENCES

Professor and Senior Leadership Position
Division of Cell Biology and Biophysics

Applications are invited for a senior leadership position in the Division of Cell Biology and Biophysics at the School of Biological Sciences, University of Missouri-Kansas City. The successful candidate should have a proven record of sustained externally funded research, scholarly activity, and leadership potential. The candidate will be expected to participate in graduate and/or undergraduate teaching, faculty mentorship, and work closely with the Dean on decision-making matters pertaining to the growth and development of the School. The School of Biological Sciences is positioning itself to become a regional leader in the areas of structural biology and molecular cell biology and welcomes applications from qualified candidates in these research areas; however, outstanding scientists from all areas of basic life sciences research are encouraged to apply. The successful candidate will receive a competitive 12-month salary, renovated research space, a start-up package commensurate with rank, and the availability of excellent research support facilities within the School of Biological Sciences. Candidates should have a Ph.D. degree and currently be in a tenured academic position at the rank of Professor.

Please direct all inquiries or nominations to **Dr. Lawrence A. Dreyfus, Dean, School of Biological Sciences (dreyfusl@umkc.edu)**. To apply, please submit electronically (MS Word or pdf) a CV, a statement of present and future research interests, and the names and addresses of 3 references to: **dreyfusl@umkc.edu**. All materials will be handled with strict confidentiality. The position will remain open until filled.

Tianjin Biochip Corporation (TBC) The company is a $20M organization with 100+ staff and located in Tianjin Economic and Development Area. Its biochip arm is one of the five bases in China supported by the State Government. It is well equipped with state-of-the-art genomics, proteomics and biochip platforms. TBC has established its core technology of molecular typing for pathogen identification and has a comprehensive IP portfolio with more than 80 granted patents. TBC also develops molecular biology reagents/kits and provides sequencing, proteomics and biochip services.

Position available:

1. VP, Business and Operation

Reporting to CEO, the VP will:

- develop and implement corporate strategic business plan
- promote company's technology, products and services globally
- be responsible for medium-to-long-term investment strategies to address technology, skill and capital requirements
- participate in annual operating plan and corporate development assessments/plan
- be responsible for ISO/GMP compliant quality systems

Minimum requirements:

- Ph.D. in molecular biology or related fields, MBA is a plus.
- 10+ years experience in a biotech organization with 5+ years management experience.

Salary is in the range of $80-100K

2. Chief Scientific Officer

Reporting to CEO, this position requires technical and managerial leadership and will:

- be responsible for all R&D and technical service activities
- develop the strategic direction of R&D
- oversee product development and technology transfer
- be responsible for setting continuous improvement goals for customer satisfaction, cost improvement and other criteria of effectiveness

Minimum requirements:

- Ph.D. in molecular biology, biochemistry, or related fields, statistical and database management experience is a plus.
- 8+ years experience in a biotech organization.

Salary is in the range of $60-100K

To Apply: Contact 86-22-66229588 Email: tbchr@tjbiochip.com

UMKC
UNIVERSITY OF MISSOURI-KANSAS CITY
SCHOOL OF BIOLOGICAL SCIENCES

Marion Merrell Dow Endowed Chair
in Biological Sciences

Applications are invited for the Marion Merrell Dow Endowed Chair in Biological Sciences at the School of Biological Sciences, University of Missouri-Kansas City. An outstanding scientist will be recruited to fill this prestigious position and lead a robust and dynamic research program. The successful candidate will also participate in graduate and/or undergraduate teaching, faculty mentorship, and work closely with the Dean and the Head of Cell Biology and Biophysics on matters pertaining to the growth and development of the School. The School of Biological Sciences is positioning itself to become a regional leader in the areas of structural biology and molecular cell biology and welcomes applications from qualified candidates in these research areas; however, outstanding scientists from all areas of basic life sciences research are encouraged to apply. The Chaired Professorship is supported by an endowment currently exceeding $3 million. The successful candidate will also receive a competitive 12-month salary, renovated research space, a start-up package commensurate with the prestige of this position, and the availability of excellent research support facilities within the School of Biological Sciences. Successful candidates should have a Ph.D. degree and currently be in a tenured academic position at the rank of Professor.

Please direct all inquiries or nominations to **Dr. Lawrence A. Dreyfus, Dean, School of Biological Sciences (dreyfusl@umkc.edu)**. To apply, please submit electronically (MS Word or pdf) a CV, a statement of present and future research interests, and the names and addresses of 3 references to: **dreyfusl@umkc.edu**. All materials will be handled with strict confidentiality. The position will remain open until filled.

Where Cures Begin

The Salk Institute for Biological Studies, a world class scientific environment and workplace located in La Jolla, CA, has an immediate opening for the position of:

Viral Vector Core Manager

The successful candidate will establish and maintain a new viral vector core facility to provide custom viral vectors supporting the work of participating scientists. The viral vector core manager will produce research-level viral vectors, including lentivirus and adeno-associated virus (AAV). Additional responsibilities include the supervision of technical staff assisting in the production of viral vectors, maintenance of plasmid libraries and cell lines, organization and maintenance.

Requirements is BS/BA in Biology or related field (PhD preferred), 3+ years of thorough experience with molecular biology and/or virology, and experience in the production of replication incompetent viral vectors. Must also have expertise in cell culture, DNA sub-cloning and transient transfection. Excellent organizational, managerial and communication skills are required; experience in the production of AAV, lentivirus and/or HSV amplicon vectors using helper virus free systems preferred.

The Salk Institute offers a highly stimulating intellectual environment and a very competitive salary and benefits program. Qualified individuals should reference job code: 9996 and apply online at www.salk.edu, or email a resume or c.v. to: correa@salk.edu. The Salk Institute for Biological Studies, 10010 N. Torrey Pines Road, La Jolla, CA 92037. EOE.

SALK INSTITUTE
FOR BIOLOGICAL STUDIES

University at Buffalo
The State University of New York

CHAIR
Department of Pharmacology and Toxicology

The School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York (UB), invites nominations and applications for the position of Professor and Chair of the Department of Pharmacology and Toxicology. The new Chair will be expected to provide the scientific vision and direction for a major expansion of the Department. Ample resources, in the form of new faculty lines and startup packages are available to implement this growth.

The Department presently includes 14 full-time, tenure-track faculty, and trains undergraduate, graduate and postdoctoral investigators. Modern, well-designed laboratory space is available, and renovation of additional research space in the Biomedical Sciences complex is scheduled to begin during the 2007 academic year. Cutting edge research cores in the areas of genomics, proteomics, microscopy/imaging, macromolecular crystallization, and transgenic animals are in place and open to all UB investigators.

The University at Buffalo is entering the second year of implementation of the UB2020 strategic plan. Departments in the School of Medicine and Biomedical Sciences play major roles in the UB2020 strengths in Molecular Recognition in Biological Systems, Bioinformatics, and Health and Wellness across the Lifespan, and the new Chair will be expected to coordinate the Department's activities with one or more of these strengths. In addition, diverse opportunities for collaboration exist at the nearby Roswell Park Cancer Institute, the Hauptman-Woodward Medical Research Institute, and the New York Center for Bioinformatics and Life Sciences. UB is the SUNY system's comprehensive campus, and the Health Sciences complex includes the Schools of Dental Medicine, Pharmacy, Public Health and Health Professions, and Nursing in addition to Medicine and Biomedical Sciences.

The successful candidate will have a Ph.D., M.D. or equivalent degree, and a well-funded and internationally recognized program of research in the broadly defined area of Pharmacology and/or Toxicology. In addition, strong leadership and administrative skills are essential attributes. Beyond maintaining a strong research program, responsibilities of the Chair include administration of the Department and its teaching programs, as well as defining its scientific vision and overall directions.

Applications, in the form of a single pdf file, should be addressed to **Dr. Kenneth Blumenthal, Chairman; Pharmacology Chair Search Committee, School of Medicine and Biomedical Sciences** and submitted to **www.ubjobs.buffalo.edu** (posting number **0601468**). Nominations or inquiries may be sent electronically to **kblumen@buffalo.edu**. Applications should be received by **August 31, 2007**, to receive full consideration.

The University at Buffalo is an Affirmative Action/Equal Opportunity Employer.

UNIVERSITY OF DELAWARE

One of the oldest institutions of higher education in this country, the University of Delaware today combines tradition and innovation, offering students a rich heritage along with the latest in instructional and research technology. The University of Delaware is a Land-Grant, Sea-Grant, Urban-Grant and Space-Grant institution with its main campus in Newark, DE, located halfway between Washington, DC and New York City. Please visit our website at **www.udel.edu.**

Jefferson Chair in Bioinformatics and Computational Biology

The College of Arts and Sciences seeks an exceptional scholar within the fields of bioinformatics and/or computational biology to fill the Edward G. Jefferson Chair. The appointment will be made at the rank of full professor with tenure. The successful candidate should have established an extensive externally-funded research program that has generated international recognition in the fields of bioinformatics or computational biology. Scholars from all relevant disciplines will be considered. The Jefferson chair will be expected to continue a high-quality independently funded research program while simultaneously leading a multi-disciplinary bioinformatics/computational research initiative comprised of faculty from biology, chemistry/biochemistry, chemical engineering, electrical and computer engineering, computer science, marine studies, physics, mathematics, animal and food sciences, plant and soil sciences, nursing and health sciences. The Jefferson Chair will lead a "cluster" recruiting effort for additional junior and mid-level faculty of appropriate disciplines within the bioinformatics and computational biology areas.

The University of Delaware houses state-of-the-art facilities for bioinformatics in the BioIT Center at the Delaware Biotechnology Institute including a compute cluster, a database cluster, and an immersive visualization studio, along with multiple specialized servers and access to a variety of analysis tools for genomics, molecular modeling, statistics and biomedical imaging. Additional details are available under **www.dbi.udel.edu/core/bioinformfacilities.html.**

Nominations and applications should be sent to Dean Tom Apple, Chair of the Jefferson Chair Search Committee, 4 Kent Way, Newark, DE 19716. A complete application will include a letter of interest, curriculum vitae, a research plan, a statement of vision for the field of bioinformatics and computational biology and a list of at least three references, with complete contact information. This position is open until filled.

The UNIVERSITY OF DELAWARE is an Equal Opportunity Employer which encourages applications from Minority Group Members and Women.

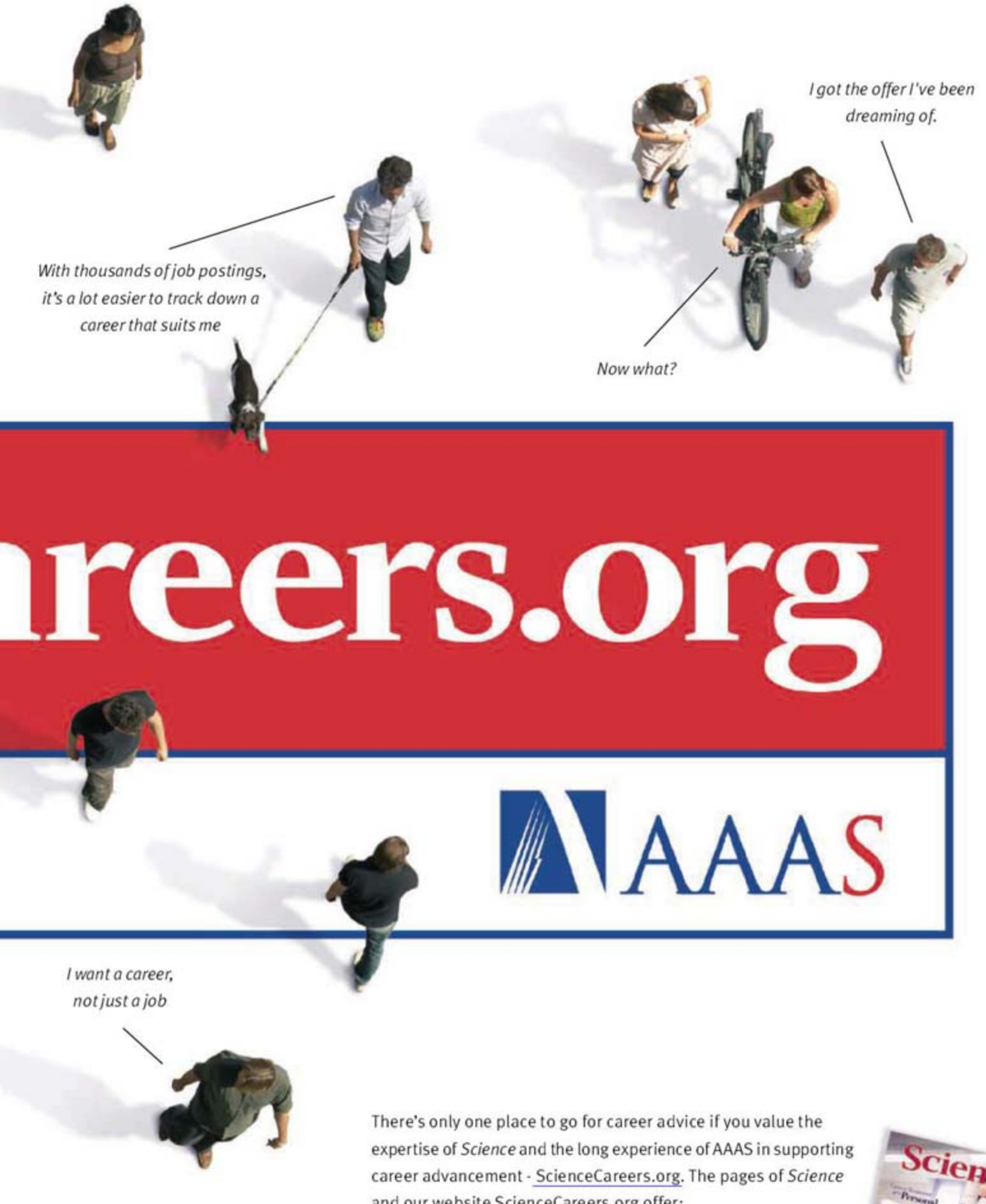
U.S. Department of Energy
Office of Science
Deputy for Programs
Announcement #SES-SC-HQ-013 (kd)

The U.S. Department of Energy's (DOE) Office of Science is seeking highly qualified candidates with outstanding scientific achievements to fill the Deputy for Programs position. The Office of Science is the single largest supporter of basic research in the physical sciences in the United States, with a 2007 budget of $3.8 billion. It oversees the Nation's research programs in high-energy and nuclear physics, basic and fusion energy sciences, and biological, environmental and computational sciences. The Office of Science is the Federal Government's largest single funder of materials and chemical sciences, and it supports unique and vital parts of U.S. research in climate change, geophysics, genomics, life sciences, and science education. The Office of Science also manages 10 world-class laboratories and oversees the construction and operation of some of the Nation's most advanced R&D user facilities, located at national laboratories and universities. These include particle and nuclear physics accelerators, synchrotron light sources, nanoscale science research centers, neutron scattering facilities, bio-energy research centers, supercomputers and high-speed computer networks. More information on the Office of Science can be found at **http://science.doe.gov**.

The Deputy for Programs provides scientific and management oversight of the six program offices by ensuring program activities are strategically conceived and executed; formulating and defending the Office of Science budget request; establishing policies, plans, and procedures related to the management of the program offices; ensuring the research portfolio is integrated across the program offices with other DOE program offices and other Federal agencies; and representing the organization and make commitments for the Department in discussions and meetings with high-level government and private sector officials. The position is within the ranks of the U.S. government's Senior Executive Service (SES); members of the SES serve in key positions just below the top Presidential appointees.

To apply for this position, please see the announcement and application instructions at **http://jobsearch.usajobs.opm.gov/ses.asp** under the vacancy announcement of **#SES-SC-HQ-013 (kd)**. Qualified candidates are asked to submit their online applications by **August 29, 2007**.

Does your next career step need direction?



There's only one place to go for career advice if you value the expertise of *Science* and the long experience of AAAS in supporting career advancement - ScienceCareers.org. The pages of *Science* and our website ScienceCareers.org offer:

- Thousands of job postings
- Career advice articles and tools
- Funding information
- Networking opportunities

www.sciencecareers.org

POSITIONS OPEN

The Office of the Vice Chancellor for Health Sciences (VCHS) and Dean of the School of Medicine of the University of California (UC), San Diego **(website: http://medicine.ucsd.edu/)** invite applications for a tenured position at the Professor level to serve as **DEAN FOR SCIENTIFIC AFFAIRS** for the Schools of Medicine and Pharmacy and Pharmaceutical Sciences. Fields of interest include, but are not limited to, biochemistry, cell biology, genetics, medicine and the medical subspecialties, microbiology/immunology, molecular biology, pharmacology, or physiology. Applicants with a broad scientific background and major scientific accomplishments will be given highest priority. Applicants must hold Ph.D. and/or M.D. degrees and will be expected to teach and supervise students, primarily at the graduate level, conduct an extramurally funded research program, and participate in administrative functions of the Health Sciences including help to set educational and space policy involving the biomedical affairs of the School, aid in recruiting leaders for the Schools, advise the VCHS and Deans on scientific matters arising, make recommendations to the graduate programs of the Schools of the Health Sciences, chair ad hoc and permanent committees dealing with the scientific affairs of the Schools, and advise the faculty in funding, scientific, and scholarly activities. Salary will be based on published UC pay scales. Review of applications will begin July 6, 2007, and will continue until position is filled. To apply, send a detailed resume, copies of selected recent publications, and names and addresses of at least three references to: **Kenneth Kaushansky, M.D., Helen M. Ranney Professor and Chair, Department of Medicine (8811), University of California, San Diego, 402 Dickinson Street, Suite 380, San Diego, CA 92103-8811.** *UCSD is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to excellence through diversity.*

CARDIAC ION CHANNELS RESEARCH

The University of Wisconsin (UW)-Madison Division of Cardiovascular Medicine seeks candidates with a Ph.D. in physiology, biophysics, or molecular biology with three to four years of postdoctoral experience to join our collaborative research efforts in aspects of cellular and molecular electrophysiology involving cardiac ion channels and cell signaling. This is a tenure-track opportunity with the UW School of Medicine and Public Health at the **ASSISTANT or ASSOCIATE PROFESSOR** level, depending on qualifications.

Outstanding diversity and quality life style is to be enjoyed in an academic medical center in a top 10 rated city. To ensure full consideration, apply with a letter of interest and curriculum vitae to:

Matthew R. Wolff, M.D.
Chief, Division of Cardiovascular Medicine
University of Wisconsin
School of Medicine and Public Health
600 Highland Avenue
G7/339 CSC (3248)
Madison, WI 53792
E-mail: mrw@medicine.wisc.edu
Website: http://www.medicine.wisc.edu

POSTDOCTORAL/STAFF SCIENTIST/ ASSISTANT PROFESSOR POSITIONS

The Molecular and Integrative Neurosciences Department at the Scripps Research Institute invites applications for Postdoctoral/Staff Scientist/Assistant Professor positions in the **Tamas Bartfai** and **Bruno Conti** Laboratories in La Jolla, California, to study central thermoregulation-energy metabolism at the level of warm sensitive neurons with molecular biological, histological tracing, and in vitro electrophysiological techniques. Two-year salaries are available for selected **MOLECULAR BIOLOGIST, NEUROANATOMIST, or ELECTROPHYSIOLOGIST**. Applicants should send their curriculum vitae, a written summary of their accomplishments, and the e-mail addresses of three references to **e-mail: bconti@scripps.edu** and **e-mail: tbartfai@scripps.edu.**

POSITIONS OPEN

INSTITUT PASTEUR

POSTDOCTORAL FELLOWSHIPS
Institut Pasteur, Paris, France

Founded in 1887 by Louis Pasteur and located in the heart of Paris, the Institut Pasteur is a world-renowned private research organization. The Pasteur Foundation of New York is seeking outstanding fellowship applicants. Candidates may apply to any laboratory within 10 Departments: Cell Biology and Infection, Developmental Biology, Genomes and Genetics, Immunology, Infection and Epidemiology, Microbiology, Neuroscience, Parasitology and Mycology, Structural Biology and Chemistry, and Virology. See website for details. Annual package is $70,000 for three years. This is a biannual call for applicants; see website for deadlines. *U.S. citizenship required.*

E-mail: pasteurus@aol.com. Website: http://www.pasteurfoundation.org.

FACULTY POSITION in PHYSIOLOGY and NEUROBIOLOGY

The Department of Physiology and Neurobiology at the University of Connecticut, Storrs, invites applications for a tenure-track faculty position available in fall 2008, at the **ASSISTANT or ASSOCIATE PROFESSOR** level. The successful candidate will be expected to maintain an independent and vigorous research program and participate in the Department's graduate and undergraduate teaching. We encourage applications from individuals studying fundamental physiological or neural processes at the molecular, cellular, or systems level. Special consideration will be given to those emphasizing membrane biology. Applicants must possess a Ph.D. and have completed at least two years of postdoctoral training. Candidates for Associate Professor are expected to have a currently funded and active research program. Review of candidates will begin on October 1, 2007, and the search will continue until the position is filled. Send curriculum vitae, a brief summary of current research with a statement of research directions, a statement of teaching interests, and the names of at least three references to: **Chair, PNB Search Committee, University of Connecticut, Department of Physiology and Neurobiology, P.O. Box U-3156, 75 North Eagleville Road, Storrs, CT 06269-3156. Website: http://www.pnb.uconn.edu.**

WASHINGTON STATE UNIVERSITY

RESEARCH ASSISTANT PROFESSOR to study the role of chromatin structure and gene expression in DNA repair in yeast, cell extracts, and mammalian cells (e.g., *DNA Repair* **4:**884, 2005; *J. Biol. Chem.* **280:**40051, 2005; *Nature Structural and Molecular Biology* **13:**902, 2006), and help train students and postdoctorals in the laboratory. Candidates should have a Ph.D. in biochemistry, molecular biology, or related field, at least two years of postdoctoral training, and the ability to communicate effectively with students and colleagues. Send statement of interests, resume, and the names of three references to: **Dr. Michael J. Smerdon, Biochemistry and Biophysics, School of Molecular Biosciences, Washington State University, Pullman, WA 99164-4660 (e-mail: smerdon@wsu.edu; telephone: 509-335-6853; fax: 509-335-9688).** Screening of applications will begin August 1, 2007. *WSU is an Equal Employment Opportunity/Affirmative Action Educator and Employer. Protected group members are encouraged to apply.*

POSITIONS OPEN

TOXICOLOGY POSITION #2007001779

The Department of Chemistry and Biochemistry at New Mexico State University (NMSU), Las Cruces, invites candidates with a Ph.D. and postdoctoral experience in toxicology, biochemistry, chemistry, pharmacology or related areas, to apply for a full-time, nine-month tenure-track position at the assistant professor level ideally beginning fall 2007. An appointment beginning a semester or so later is possible. The successful candidate is expected to develop a nationally recognized and externally funded (NIH, NSF, USDA, Department of Defense, et cetera) research program in biochemical/molecular/bioanalytical toxicology, teach core courses at the undergraduate and graduate levels in toxicology, and contribute to the training of undergraduate and graduate students in toxicology and related fields. Candidates with expertise in forensic toxicology and research areas that complement existing faculty interests are encouraged to apply. Applicants must submit copies of undergraduate and graduate course transcripts, curriculum vitae, three letters of reference, a brief description of proposed research, a statement of teaching philosophy, and representative reprints of published research. For more information see **website: http://www.chemistry.nmsu.edu/Toxicology.html.** Send applications to:

Dr. Glenn D. Kuehn
Toxicology Faculty Search Committee
New Mexico State University
Department of Chemistry and Biochemistry
MSC 3C, P.O. Box 30001
1175 N. Horseshoe Drive
Las Cruces, NM 88003-8001

Review of applications will begin July 16, 2007, and will continue until the position is filled. *New Mexico State University is an Equal Employment Opportunity/Affirmative Action Employer. Men and women, and members of all racial and ethnic groups, are encouraged to apply. Offer of employment is contingent upon verification of individual's eligibility for employment in the United States and verification of certain credentials, criminal, and other background information. NMSU is a public, land grant, and minority-serving institution recognized by the Carnegie Foundation for Advancement of Teaching as a Doctoral/Research University-Extensive.*

ASSISTANT PROFESSOR of CHEMISTRY/ BIOCHEMISTRY

Eastern Connecticut State University. Full-time tenure-track position in chemistry. Ph.D. in chemistry, biochemistry, or related field required. Strong evidence of quality teaching and postdoctoral experience preferred. Teaching expectations include advanced chemistry and biochemistry courses, emphasis on instrumentation and biochemical techniques, as well as introductory chemistry courses to augment the integrative liberal arts core curriculum. The successful candidate will support the Department's growing Biochemistry Program through course development, program assessment, and independent research involving undergraduates. Applicants should send curriculum vitae, transcripts, statements of teaching philosophy, research interests, and three current letters of recommendation to: **John Toedt, Search Chair, Department of Physical Sciences, 83 Windham Street, Willimantic, CT 06226.** *Eastern Connecticut State University is an Equal Opportunity/Affirmative Action Employer.*

A POSTDOCTORAL RESEARCH ASSOCIATE POSITION is immediately available for a qualified Ph.D. or M.D. to study integrin-mediated signalling involved in the remodeling of adrenergic regulation of calcium channels in cardiac myocytes. Experience with single cell voltage clamp techniques is required and confocal fluorescence microscopy is desirable. Send curriculum vitae, names and addresses of three references, and a brief statement of research experience and interests to: **Dr. Stephen L. Lipsius, Department of Physiology, Loyola University Chicago, 2160 South First Avenue, Maywood, IL 60153. Fax: 708-216-2606, e-mail: slipsiu@lumc.edu.**

POSITIONS OPEN

The College of Sciences and Mathematics at Auburn University located in Auburn, Alabama **(website: http://www.auburn.edu/cosam)**, is seeking candidates for the position of **POSTDOCTORAL FELLOW** in the sciences and mathematics. From time to time, Postdoctoral positions become available under a variety of research grants and projects in the College. We are seeking applications from individuals with a Ph.D. in any one area such as: biology, chemistry, geology, geography, mathematics, statistics, physics, or related fields. *Selected candidates must meet eligibility requirements to work in the United States at the time the appointment is scheduled to begin and must be able to communicate effectively in English.* The positions are available for a minimum of one year as full-time 12-month, with renewal possible. Salary will be commensurate with education and experience. Review of applications will begin after July 1, 2007, and continue throughout the year as positions become available. Please send curriculum vitae, a statement of research interests, along with a list of three references and contact information to: **Dr. Marie Wooten, Associate Dean for Research, College of Sciences and Mathematics, 315 Roosevelt Concourse, Auburn University, AL 36849. E-mail: wootemw@auburn.edu; fax: 334-844-5255.** *Minorities and women are encouraged to apply. Auburn University is an Affirmative Action/Equal Opportunity Employer.*

Dade Behring has an immediate need for a **CONJUGATION SCIENTIST** at our Newark facility: The Conjugation Scientist will: (1) Design and synthesize bio-conjugates as part of R&D effort for in vitro diagnostics. (2) Advance the knowledge base and competencies within group of organic and bioconjugation specialists. Patent novel processes and compositions. (3) Work closely with and support developers of diagnostic assays to understand and help resolve technical problems. (4) Supervise and help advance the skills of a supporting assistant or associate chemist. (5) Report results in writing and oral presentations. Qualified candidate will have: (1) Ph.D. in chemistry with strong background in protein chemistry and synthetic organic chemistry. (2) One to two years of experience in bio-conjugation. (3) Excellent communication skills, both written and oral. (4) Ability and willingness to work as team member. (5) Familiarity with contemporary scientific literature in organic and biochemistry. This job works with controlled substances. *A criminal background check and Drug Enforcement Agency (DEA) screening is required.* If you meet the requirements for this position and would like to apply, send your resume and cover letter with salary requirements to **e-mail: dade_chemistry_systems@dadebehring.com** or **fax: 302-631-0348.** Please reference 07DE150.

FACULTY POSITION
Mucosal Immunology

Food Animal Health Research Program, Ohio Agricultural Research and Development Center (OARDC), the Ohio State University (OSU), in Wooster, Ohio, seeks applications for one of several new faculty positions related to the OSU Targeted Investment in Excellence Program in Public Health Preparedness. This opportunity is at the **ASSISTANT or ASSOCIATE PROFESSOR** level, for a research-intensive, tenure-track position in mucosal immunology with application to preharvest food safety. Candidates must have a D.V.M., or equivalent degree, and/or Ph.D. degree and proven expertise in the conduct of hypothesis-driven research, current immunological methods, and knowledge of zoonotic diseases desired. Please submit curriculum vitae, a statement of research interests, and names of three references to: **Dr. Jeffrey LeJeune, Faculty Search Chair, Food Animal Health Research Program, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691; e-mail: lejeune.3@osu.edu.** Review of applications begins July 31, 2007. For more information, see **website: http://www.oardc.ohio-state.edu/fahrp.** *OSU is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

Applications are invited for the position of **CHAIR of the DEPARTMENT of BIOLOGY** at Northeastern University **(website: http://www.biology.neu.edu/index.html).** The successful candidate will lead and expand an evolving Department that plays a key role in the University. We are seeking nationally recognized leaders who have an on-going record of competitive research funding. The Biology Department has 28 faculty. Research interests in the Department are diverse and span the spectrum from organismal to molecular studies. The successful candidate will complement these areas or will bring his/her own theme and cluster of key hires. We are especially interested in an individual who will foster and expand collaborative and interdisciplinary research. Existing interdisciplinary initiatives include biotechnology, neurobiology, sensing and imaging, and nanotechnology, with participants from chemistry and chemical biology, physics, engineering, and the College of Health Sciences. As part of the University's continuing growth as a research institution, Northeastern is in the process of hiring 30 outstanding faculty to pursue interdisciplinary research and teaching. A competitive startup package will be provided.

Please send a letter of application, curriculum vitae, and a brief description of research interests. The earliest start date will be spring of 2008, and the search will continue until the position is filled. Applications should be submitted electronically to **e-mail: biojobs@neu.edu.**

Northeastern University is an Equal Opportunity/Affirmative Action Employer. Candidates from groups underrepresented in science are especially encouraged to apply.

USDA, Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), in Raleigh, North Carolina, is recruiting for a Senior Executive Service (SES) position, **DIRECTOR, CENTER for PLANT HEALTH SCIENCE and TECHNOLOGY.**

In this position, you will represent the Deputy Administrator with responsibility for the overall planning, coordination, and direction of development and transfer of technology used in APHIS plant health programs.

In addition, you will provide national leadership in developing and delivering new science and technology into operational program activities.

You will lead a complex and diverse organization of professional, technical, and administrative staff of 250 employees assigned to headquarters and eight laboratories. You must possess a degree in biological sciences, agriculture, natural resource management, chemistry, or related disciplines appropriate to the position, and must have supervisory/managerial experience which demonstrates the ability to provide leadership to a complex, service-oriented organization and its resources.

The salary range for this position is $111,676 to $154,600.

To apply, contact **Jocelyn White** at **telephone: 202-720-3010** for information about the position and instructions on how to apply.

Please reference vacancy announcement number APHIS-SES-07-02A, and note the closing date of April 16, 2007. Only complete applications received by the closing date will be accepted.

POSTDOCTORAL POSITION available starting August 2007, to assess impact of multiple factors (deer, invasive earthworms, invasive plants, invasive invertebrates) on demography of native forest plants. Interest and experience in plant-herbivore manipulations and plant demography desirable.

For further inquiries or to submit an electronic application (statement of research interests, curriculum vitae, and names of three references) please contact: **Dr. Bernd Blossey, Department of Natural Resources, Cornell University, Ithaca, NY 14853 (e-mail: bb22@cornell.edu).**

POSITIONS OPEN

POSTDOCTORAL POSITIONS

Two positions available immediately focused on the interactions of influenza viruses with host cells. Project One involves a structure/function analysis of the viral hemagglutinin and the conformational changes leading to fusion activation for avian, equine, and human influenza viruses. For this project, strong molecular biology skills are required, combined with a background in biophysical approaches to protein structure. This project is associated with the newly established National Institute of Allergy and Infectious Diseases Centers of Excellence for Influenza.

Project Two involves a characterization of the signaling responses controlling virus endocytosis, with a focus on polarized epithelial cells. For this project, a background in molecular cell biology is required.

Interested individuals holding appropriate Ph.D.s should send curriculum vitae, along with three professional references, to: **Dr. Gary Whittaker (e-mail: grw7@cornell.edu), Department of Microbiology and Immunology, Cornell University College of Veterinary Medicine, Ithaca, NY 14853.**

POSTDOCTORAL OPPORTUNITY

An exciting opportunity is available for a driven postdoctoral candidate to study novel aspects of anti-viral innate immunity, interferon signaling, and virus-host interactions. Potential projects will include dissecting innate immune signal transduction pathways and analyzing the functions of novel genes in vitro and in vivo. The ideal applicant will be well-trained in molecular biology and mammalian cell culture techniques, have good communication skills, and possess at least two first-author papers in reputable journals. Please see the following publications for examples of our work: *Journal of Immunology* **178(4):**2429-39, 2007, *Nature* **432(7015):**401-5, 2004, *Cancer Cell* **(5)1:**51-65 2004, *Immunity* **13(1):**129-41, 2000.

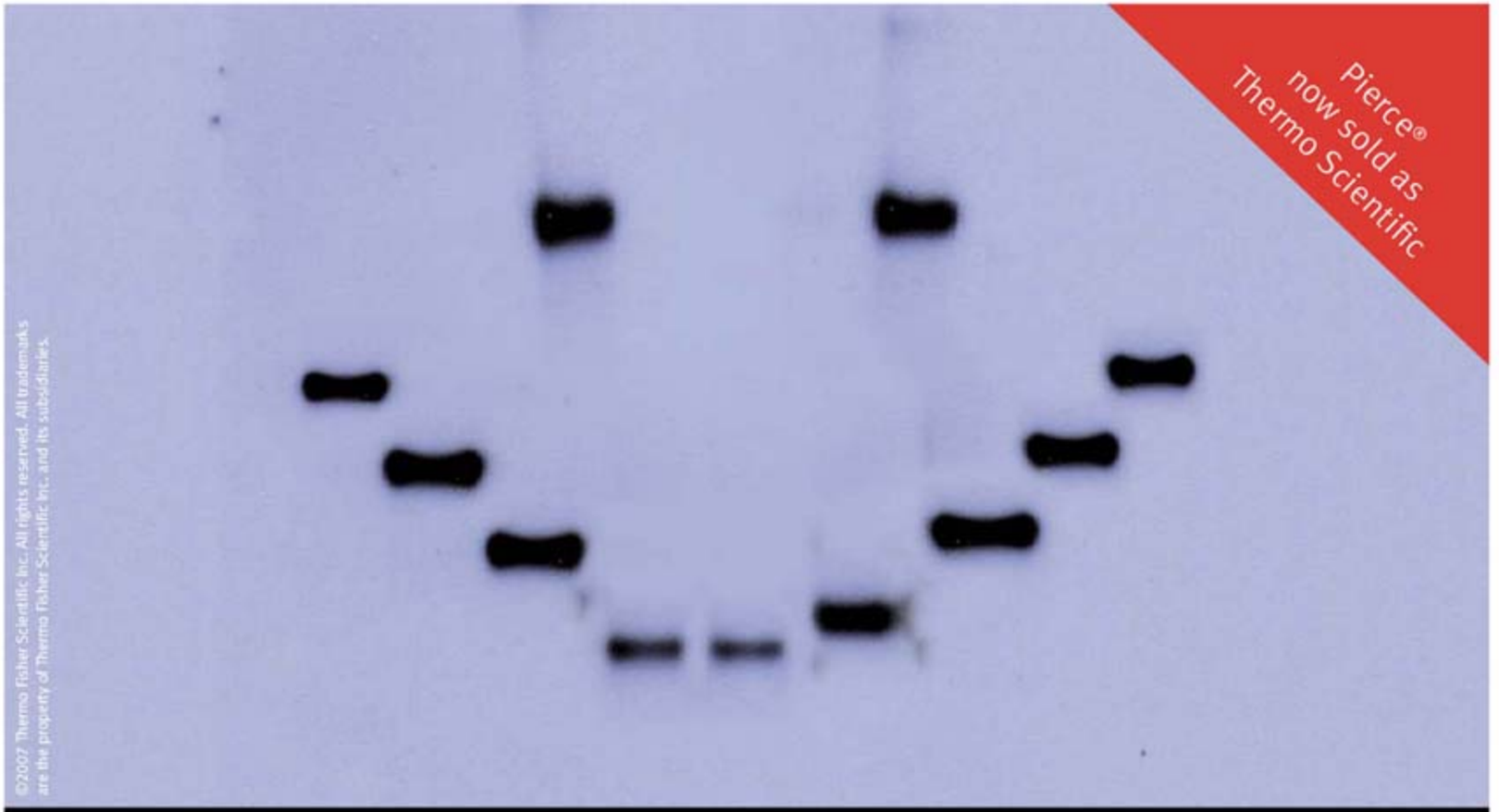
Please forward curriculum vitae to: **Siddharth Balachandran, Associate Member, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111** or **e-mail: sid.balachandran@fccc.edu.** *Equal Opportunity Employer.*

AWARDS

RANBAXY RESEARCH AWARDS 2006

The last date for submitting nominations for Ranbaxy Research Awards 2006 has been extended to 31 July 2007. Our new **website: http://www.ranbaxysciencefoundation.org.**

MARKETPLACE



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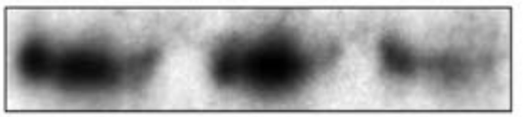

100 fg 10 fg 1 fg

True femtogram detection of IκBα using Thermo Scientific SuperSignal Femto Substrate. Serially diluted samples were run on Precise™ Precast Gels, transferred to PVDF membrane and blocked with StartingBlock™ Blocking Buffer. The blot was then incubated in rabbit anti-IκBα followed by incubation in goat anti-rabbit HRP. The substrate was added and the membrane was exposed to CL-XPosure™ Film. For complete details visit our website.

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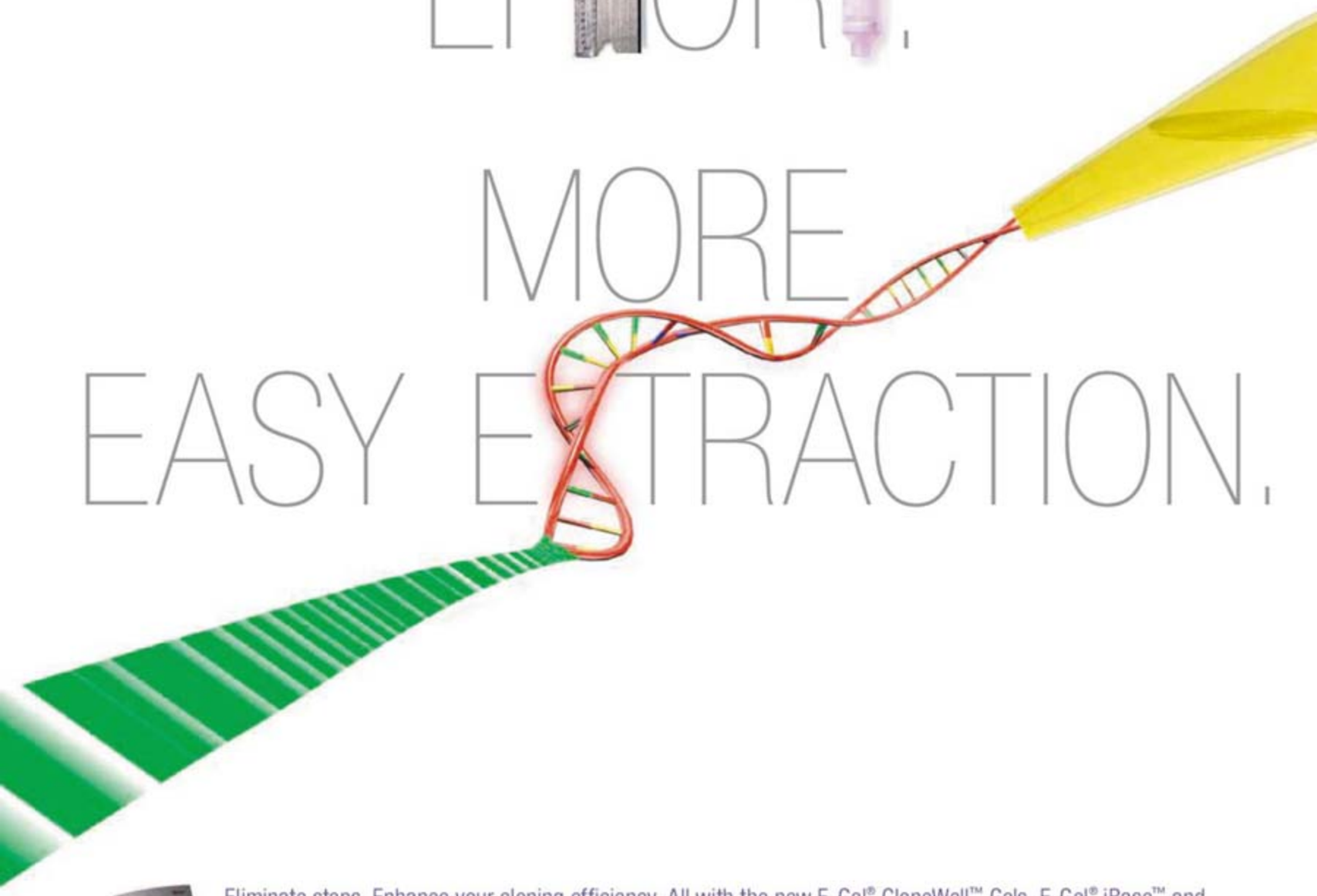

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